

Demographic history of two endangered Atlantic eel species, *Anguilla anguilla* and *A. rostrata*

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Research Article

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Abstract

European (*Anguilla anguilla*) and American eel (*A. rostrata*) are panmictic catadromous fish species, which have experienced recent drastic population declines amounting to just a few percent recruitment relative to levels prior to 1980. However, little is known about the extent of recent population declines relative to historical fluctuations. We analyzed demographic histories of the species using a method for reconstructing skyline plots based on site frequency spectra, in this case derived from restriction site-associated DNA (RAD) markers. The results showed very high effective population sizes ranging in the millions for most of the time range covered. Both species experienced ancient declines coinciding with the time of speciation (ca. 170,000 generations ago) and at a later stage where secondary contact occurred (ca. 90,000 generations ago). Whereas the demographic histories of the species were similar most of the time, they followed widely different trajectories from ca. 70,000 to 40,000 generations ago. However, for the past ca. 30,000 generations both species have shown demographic stability, even across glacial and interglacial periods. We discussed the possible environmental factors, including ocean current changes and geomagnetism reversal that could have affected demographic history and further suggest that southward displacement of spawning regions and continental distribution could explain the apparent stability even during glaciations. The recent declines appear unprecedented against a backdrop of long-term demographic stability, underpinning concerns that low density of spawners in the huge spawning region could lead to detrimental Allee Effects.

Introduction

Anthropogenic global change poses a range of environmental problems that profoundly affect populations, species and biodiversity world-wide (Anon. 2005). It is well established that rare species showing limited geographical distributions are more prone to becoming endangered (Gaston 1998; Loiseau et al. 2020). It is therefore all the more worrying when even previously wide-spread species with high population sizes show catastrophic declines or go extinct. A classic example concerns the passenger pigeon (*Ectopistes migratorius*), which exhibited population sizes in the billions but nevertheless went extinct over just a few decades, due to hunting and habitat destruction (Hung et al. 2014; Murray et al. 2017). Reconstruction of its demographic history using genomic data has suggested that the species would historically exhibit strong population fluctuations, and anthropogenic pressure could have coincided with a cyclic population decline (Hung et al. 2014), although this result has been disputed (Murray et al. 2017).

A more recent example of widespread species experiencing drastic population declines concerns the two sister species of Atlantic eels, European (*Anguilla anguilla*) and American (*A. rostrata*) eels. They both spawn in thermal fronts of the Southern Sargasso Sea, from where the larvae are advected by ocean currents to continental coasts and inland rivers and lakes, ranging from Morocco to Iceland and the White Sea region (European eel) and Venezuela to Greenland (American eel) (Tesch 2003). Adults migrate back to the Sargasso Sea to spawn and presumably die (Tesch 2003; Aarestrup et al. 2009). Both species are remarkable by being panmictic (Dannewitz et al. 2005; Als et al. 2011; Côté et al. 2013; Pujolar et al. 2014;

Enbody et al. 2021). They have experienced recent drastic declines, ascribed to overfishing, pollution, habitat destruction, introduced parasites and climate change, with recruitment of juvenile eels having declined by 90–99% in European eel relative to pre-1980 levels and commercial catches of adult American eels declining by 50% in some regions over the same time period (Drouineau et al. 2018). They are currently listed as “Critically Endangered” (European eel) and “Endangered” (American eel) in IUCN’s (International Union for the Conservation of Nature) Redlist (<https://www.iucnredlist.org>).

Estimates of historical effective population sizes (N_e) of both species, obtained using genomic data, range in the hundreds of thousands or more (Pujolar et al. 2013; Nikolic et al. 2020), and census population sizes are expected to be orders of magnitudes higher, depending on N_e/N ratios (Palstra & Ruzzante 2008). Hence, despite recent drastic declines, this is not expected to leave a detectable genomic imprint in the short term and to result in numerically small N_e values leading to e.g. inbreeding (Frankham et al. 2014). However, given the vast extent of the spawning areas, ranging ca. 2,000 km from east to west for European eel (Miller et al. 2019), declines could result in inability of spawners to find each other and aggregate, leading to catastrophic Allee effects (Stephens et al. 1999; Hutchings 2015) long before genetic problems could be an issue. It would therefore be highly relevant to assess if the two species have previously experienced natural declines comparable to the present anthropogenic declines, and if current declines coincide with general long-term cyclic declines.

Several methods have been developed for reconstructing demographic history based on genomic data (Beichman et al. 2018). These include model-constrained methods that use site frequency spectra (SFS) to test the likelihood of a given demographic model (Gutenkunst et al. 2009; Excoffier et al. 2013). In recent years, several model-flexible methods have also been developed to infer substantially more detailed demographic history, without the need for specifying the specific demographic model (Li & Durbin 2011; Sheehan et al. 2013; Liu & Fu 2015; Terhorst et al. 2017; Liu & Fu 2020). Whereas most of these require whole genome sequences and have issues with resolving more recent demographic history, the Stairway Plot 2 method uses folded or unfolded SFS and has been found suitable for inferring more recent population history than most other methods (Liu & Fu 2020).

The methods PSMC (Li & Durbin 2011) and $\delta a\delta i$ (Gutenkunst et al. 2009) have previously been used for inferring population and speciation history of Atlantic eels (Nikolic et al. 2020). The results from both methods were congruent but with limited resolution towards the present, although suggesting long-term population growth. In the present study, we reanalyzed previously published RAD (Restriction site Associated DNA) sequencing data from European and American eel (Jacobsen et al. 2014a; Pujolar et al. 2014). We aligned reads to a newly available chromosome-level assembly of the European eel genome (Rhie et al. 2021) and analyzed demographic history using Stairway Plot 2 (Liu & Fu 2020). Our aims were to analyze historical fluctuations of N_e in both species and identify possible environmental determinants, and to assess if the current declines are unprecedented or if they have parallels to regularly occurring events, e.g. associated with glacial cycles.

Material And Methods

Samples and data

The samples used in this study included 172 European eels from six locations (Burrishoole and Lough Erne, Ireland, Canet and Gironde, France, Ringhals, Sweden, and Valencia, Spain) and 30 American eels from four locations (Rivière Blanche and Mira River, Canada and St. John's River, USA), sampled between 1999 and 2010. For more specific details we refer to the original papers (Jacobsen et al. 2014a; Pujolar et al. 2014). Sequencing libraries in these studies were prepared following the standard RAD protocol (Baird et al. 2008) and using the restriction enzyme *EcoRI* and sequenced (90 bp paired-end) on an Illumina Genome Analyzer II by Beijing Genomics Institute (BGI, Hong Kong, China).

Data processing

The samples from each species were pooled, as both species are assumed to be panmictic. Only the first (left) paired-end reads were used for data analysis. The raw reads were filtered using fastp 0.20.0 (Chen et al. 2018) (with parameters -q 20 -u 0 -l 75 -b 75) to eliminate reads with low quality and trim the read lengths to 75 bp to avoid sequencing errors (Pujolar et al. 2013). The clean reads were aligned to the recent chromosome-level European eel reference genome (assembly GCA_013347855.1) (Rhie et al. 2021) using BWA-MEM (Li & Durbin 2009). The resulting SAM files were coordinate-sorted and converted to BAM files using samtools v1.9 (Li et al. 2009). The *gstacks* program in Stacks version 2.59 (Rochette et al. 2019) was used to identify and genotype SNPs for each locus and individual, and the *populations* program in Stacks was used for exporting a standard VCF (variant call format) genotype file (parameters -p 2 -r 0.9, excluding loci with > 10% missing data). Putative hybrids between the two species were identified using ADMIXTURE (Alexander & Lange 2011). After removing hybrids, the program *populations* (-p 1 -r 0.9) was conducted again to generate a standard VCF file for each species separately.

Analysis of demographic history

The VCF file was subsequently filtered to remove loci with exceptionally low or high coverage or deviation from Hardy-Weinberg equilibrium (HWE) using VCFtools (parameters -min-meanDP 5 -max-meanDP 40 -hwe 0.0001) (Danecek et al. 2011). Folded SFS for each species were generated from the filtered VCF file by the R script *vcf2sfs* (Liu et al. 2018). The demographic history was inferred by Stairway plot 2.1 (Liu & Fu 2020) using the following parameters: the singletons were ignored to avoid possible bias due to sequencing errors; mutation rate per site per generation was assumed to be 1e-8; we assumed a generation length of European eel of 13 years. Generation length is complicated to estimate in Atlantic eels due to growth differences and resulting differences in age at maturity across geographic regions (Tesch 2003), combined with difficulties in assessing the contribution of eels from different regions to the total spawning population. Moreover, it is possible that generation length differs between the two species. Hence, the IUCN estimate of generation length is 13 years in European eel (<https://www.iucnredlist.org/species/60344/152845178>) and 12 years in American eel (<https://www.iucnredlist.org/species/191108/121739077>). We therefore assumed the 13 year generation

length in European eel and used the inferred time of speciation (150,000-160,000 generations ago as per Nikolic et al. (2020)) to calibrate generation length in American eel under the assumption that demographic history should be similar prior to this time. We a

Results And Discussion

SNP data

A total of 3.06 million to 15.98 million clean reads passed the data filtering for each sample with an average value of 6.66 million. Among them, 95.17–98.38% were successfully mapped to the reference genome. The Stacks analysis for all samples generated 300,683 RAD loci, the number of which is almost twice that of estimated recognition sites (160523) of *EcoRI* in the reference genome (two RAD loci should be associated with each restriction site). One admixed individual was identified and removed from the American eel population. A total of 4,311,758 SNPs from 328,631 RAD loci representing 26,229,220 bp were identified in European eel, and 1,829,324 SNPs from 318,424 RAD loci representing 25,414,634 bp were obtained from American eel. After removing SNPs with extreme coverage or deviation from HWE, 4,214,493 and 1,817,481 SNPs remained with an average depth per site of 15.9 and 17.5 for European and American eel, respectively. The values of genome-wide nucleotide diversity (π) were 0.0060 in European eel and 0.0068 in American eel, comparable to estimates reported by Nikolic et al. (2020), also showing slightly higher π in American eel.

Demographic history reconstruction

The folded SFS of European eel and American eel are shown in Fig. 1, both of which exhibit clear L-shapes. Demographic histories of the two species, inferred using Stairway Plot 2 are shown in Fig. 2. Assuming a generation length of 13 years for European eel, the demographic history coinciding with and prior to the presumptive time of speciation was aligned for the two species when a generation length of 10.7 years was assumed for American eel. Conversely, assuming the same generation length for both species led to shifted timing of demographic histories (see Supplementary Information Fig. S1).

Compared to PSMC analysis (Li & Durbin 2011) based on individual genomes (Nikolic et al. 2020), Stairway Plot 2 provides higher resolution and more detailed histories. The time range of histories for both species encompasses ca. 5.00 million years ago (Mya) to the present. The historical N_e values since species divergence are estimated to range from 39,769 to 3,984,743 for European eel, and from 49,793 to 4,999,485 for American eel, with the maximum being about 100 times higher than the minimum for both species. With one notable exception the trajectories for both species are quite similar. Hence, N_e appears stable until ca. 170,000 generations ago, when a decline occurs that represents the minimum N_e for both species. This is followed by an increase of N_e starting ca. 160,000 generations ago. The time of this event corresponds well with the divergence time and hence time of speciation (150,000-160,000 generations ago) inferred using $\delta a \delta i$ (Gutenkunst et al. 2009) in Nikolic et al. (2020). The second major event involves strong declines starting ca. 90,000 generations ago. This corresponds to the time (87,000–92,000

generations) of onset of secondary contact between the species, inferred using $\delta a\delta i$ (Nikolic et al. 2020). Subsequently, N_e of American eel increases strongly and remains high and stable towards the present. European eel, in contrast, shows a much lower increase, remains stable from ca. 70,000 to 40,000 generations ago, possibly with a decline towards the end of this interval. From ca. 40,000 generations ago towards the present, N_e has remained stable after a drastic expansion.

Environmental factors influencing demographic history

Nikolic et al. (2020) provided evidence for speciation of Atlantic eels involving vicariance, followed by isolation and later secondary contact. Our analyses show that both divergence and secondary contact coincide with periods of population decline. Declines at the time of divergence could simply reflect a previously common gene pool splitting into two, whereas the declines at the onset of secondary contact are more surprising; intuitively it could be expected that strong population growth rather than declines would lead to more overlap of spawning regions and hence secondary contact. The life cycle of Atlantic eels is intimately linked to ocean currents, particularly the Gulf Stream (Tesch 2003). It has been suggested that initial divergence of the species was due to ocean current changes leading to isolation of the spawning regions (Avice et al. 1990; Nikolic et al. 2020), possibly mediated by Heinrich Events; massive discharges of icebergs during glaciations, leading to decreased salinity and weakening of the Gulf Stream (Heinrich 1988; Hodell et al. 2008). The second decline could again result from Heinrich Events which caused ocean current changes bringing the spawning regions back into contact. In contrast, the subsequent time period with highly different demographic histories of the species is more likely to reflect factors influencing one species and not the other, hence not involving ocean current changes. Geomagnetism is considered highly important for navigation of eels during their larval spawning migrations (Durif et al. 2021), and hence changes to the Earth's magnetic fields could have negative effects. The Brunhes-Matuyama magnetic reversal occurred ca. 780,000 years ago (Bassinot et al. 1994), which roughly coincides with the time of divergent demographic histories of the species (Fig. 2). If this has negatively affected Atlantic eels, the effect would be expected to be stronger for European eel (with spawning migration > 5,000 km) compared to American eel (spawning migration > 1,500 km). We note, however, that this event does not entirely overlap with the time span during which the species show different demographic histories, and it can at most be one of several factors involved. Other, as yet unidentified factors affecting the European but not North American continent are also possible.

Based on analysis of mitogenome sequences, it has been suggested that speciation of Atlantic eels occurred ca. 3.38 Mya, coinciding with the closure of the Panama Gateway (Jacobsen et al. 2014b). However, there are no imprints in the demographic histories coinciding with this time (Figure. 1). Strikingly, there are also no discernible demographic effects of cycles of glacial and interglacial periods and even relatively recent drastic events like the Last Glacial Maximum (Fig. 2), as otherwise evident in demographic histories inferred in a range of organisms including freshwater fishes, birds and mammals using PSMC (Moura et al. 2014; Liu et al. 2016; Nadachowska-Brzyska et al. 2016; Pujolar et al. 2017; Mays et al. 2018). The result may seem counterintuitive, given that subfossil data suggests that European eel was absent from the northern part of its present distributional range during the Last Glacial

Maximum (Kettle et al. 2008), and similar patterns are expected for American eel. An explanation could be that the Gulf Stream was displaced further South during glaciations, and juvenile eels could have been swept towards more southern regions of North Africa and South America (Kettle et al. 2008), leading to different distributional ranges but without population declines.

Current declines in the context of historical fluctuations

Our results suggest that the current declines of European and American eels do not coincide with general historical trends of declines. On the contrary, both species are suggested to have been relatively stable for at least the last hundreds of thousands of years, and the current declines seem unprecedented in the species' history. For instance, the 90–99% decrease in European eel recruitment only find parallels when considering events that occurred > 2 Mya. However, such conclusions should of course be treated with caution. First, our results depend on assumptions of generation length and mutation rate, but it would require unrealistic changes of these to lead to qualitatively different conclusions regarding timing of events, and relative magnitudes of N_e through time would remain the same. Second, current declines are estimated as census population sizes (N) encompassing juvenile recruitment or spawning escapement, whereas estimated demographic histories are based on N_e . Comparison of trends at N and N_e assumes a constant ratio between the two, but there is evidence for increased N_e/N ratios in some cases of declining populations (Palstra & Ruzzante 2008), possibly reflecting density-dependent effects. However, there is virtually no knowledge of N_e/N ratios and their constancy or variation in eels. Third and finally, methods like Stairway Plot 2 capture major trends of demographic history and would not be likely to detect fluctuations occurring at the scale of decades or centuries. Nevertheless, the population dynamics and especially long generation lengths of Atlantic eels mean that recovery from low population sizes will be slow. Hence, it has been shown that even if all fishing for European eel was abandoned it would still take 80 years to achieve recovery comparable to pre-1980 levels (Astrom & Dekker 2007). This increases the possibilities that drastic historical declines, if they had occurred, would last sufficiently long to leave detectable imprints in genomes.

In total, our results suggest that the current drastic declines of European and American eel occur against a backdrop of long-term stability. Hence, the species may now be entering uncharted territories, and concerns about low densities of spawners in the huge spawning region resulting in Allee effects must be considered very real.

Declarations

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Data accessibility Sequence data were derived from previous studies (Jacobsen et al. 2014a; Pujolar et al. 2014), deposited in the NCBI Sequence Read Archive under project numbers PRJNA195555 and PRJNA230782.

Author contributions M.M.H., X.F. and S.L. designed and conceived the project. X.F. and S.L. performed data analysis. X.F. and M.M.H. wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests The authors declare no competing interests.

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Figures

Figure 1

The folded Site Frequency Spectrum of European eel (a) and American eel (b).

Figure 2

Demographic histories of Atlantic eels inferred by Stairway Plot 2, assuming generation length of 13 years for European eel (*Anguilla anguilla*) and 10.7 for American eel (*A. rostrata*). The solid line and dotted lines indicate the median estimate and the 2.5 (upper) and 97.5 (lower) percentile estimation of the effective population size, respectively. The gray shadows from left to right indicate five historical events: the last glacial maximum (LGM), the Brunhes-Matuyama magnetic reversal, speciation and secondary contact between the two species inferred using $\delta a \delta i$ (Nikolic et al. 2020), and the closure of the Panama Gateway. Inferred demographic histories assuming the same generation length for both species are shown in Supplementary Material Fig. S1.

Supplementary Files

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