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*Published in:*  
Molecular Ecology Resources

*Link to article, DOI:*  
[10.1111/1755-0998.12117](https://doi.org/10.1111/1755-0998.12117)

*Publication date:*  
2013

[Link back to DTU Orbit](#)

### *Citation (APA):*

Pujolar, J. M., Jacobsen, M. W., Frydenberg, J., Als, T. D., Larsen, P. F., Maes, G. E., ... Hansen, M. M. (2013). A resource of genome-wide single-nucleotide polymorphisms generated by RAD tag sequencing in the critically endangered European eel. *Molecular Ecology Resources*, 13, 706-714. <https://doi.org/10.1111/1755-0998.12117>

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## A resource of genome-wide single nucleotide polymorphisms generated by RAD tag sequencing in the critically endangered European eel

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### ABSTRACT

Reduced representation genome sequencing such as RAD (Restriction-site Associated 2 DNA) sequencing is finding increased use to identify and genotype large numbers of 3 single nucleotide polymorphisms (SNPs) in model and non-model species. We 4 generated a unique resource of novel SNP markers for the European eel using the 5 RAD sequencing approach that were simultaneously identified and scored in a 6 genome-wide scan of 30 individuals. Whereas genomic resources are increasingly 7 becoming available for this species, including the recent release of a draft genome, no 8 genome-wide set of SNP markers was available until now. The generated SNPs were 9 widely distributed across the eel genome, aligning to 4,779 different contigs and 10 19,703 different scaffolds. Significant variation was identified, with an average 11 nucleotide diversity of 0.00529 across individuals. Results varied widely across the 12 genome, ranging from 0.00048 to 0.00737 per locus. Based on the average nucleotide 13 diversity across all loci, long-term effective population size was estimated to range 14 between 132,000 and 1,320,000, which is much higher than previous estimates based 15 on microsatellite loci. The generated SNP resource consisting of 82,425 loci and 16 376,918 associated SNPs provides a valuable tool for future population genetics and 17 genomics studies and allows for targeting specific genes and particularly interesting 18 regions of the eel genome.

*Keywords:* effective population size; population genomics; RAD sequencing; SNP discovery

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**Article first published online: 9 MAY 2013**

Please note that this is an author-produced PostPrint of the final peer-review corrected article accepted for publication. The definitive publisher-authenticated version can be accessed here:

<http://dx.doi.org/10.1111/1755-0998.12117> © 2013 John Wiley & Sons Ltd

## 1 **Introduction**

2 Recent advances in the speed, cost and accuracy of next-generation sequencing  
3 technologies are revolutionizing the field of population genetics and facilitating the  
4 application of genomic approaches into ecological and evolutionary studies (Allendorf  
5 *et al.* 2010; Davey *et al.* 2011). The growing accessibility to high-throughput  
6 sequencing methods allows the production of extremely large collections of data and  
7 the discovery of genome-wide resources at relatively modest and decreasing costs.  
8 Although ecological and evolutionary genomic studies involving the complete  
9 sequencing of multiple individuals and/or populations are still costly and have been  
10 restricted to few organisms (Jones *et al.* 2012a), genotyping-by-sequencing  
11 approaches (i.e. sequencing of a reduced representation of the genome followed by  
12 single-nucleotide polymorphism (SNP) discovery) can provide data on hundreds of  
13 thousands of SNPs that are to some extent evenly distributed across the genome.

14 One such genotyping-by-sequencing approach is the use of high-throughput  
15 sequencing of Restriction-site Associated DNA tags (RADs) (Miller *et al.* 2007; Baird *et al.*  
16 *et al.* 2008). RAD tags are short fragments of DNA adjacent to each instance of a  
17 particular restriction enzyme recognition site. Different RAD tag densities can be  
18 achieved by choice of restriction enzyme. By focusing sequencing efforts only on those  
19 tags flanking a restriction site in multiplexed individually-barcoded samples, RAD  
20 sequencing allows efficient high-density identification of SNPs. Recently, a number of  
21 related genotyping-by-sequencing methods have been developed, including double-  
22 digest methods that considerably simplify library construction but generally also  
23 provide less coverage of the genome as compared to the original RAD method (Elshire  
24 *et al.* 2011; Peterson *et al.* 2012; Bruneaux *et al.* 2013). Different types of  
25 genotyping-by-sequencing approaches have been successfully used to discover  
26 thousands of SNPs in fish (Hohenlohe *et al.* 2010; 2011; Bruneaux *et al.* 2013),  
27 mammals (Peterson *et al.* 2012), insects (Emerson *et al.* 2010) and plants (Barchi *et al.*  
28 *et al.* 2011; Scaglione *et al.* 2012). Hence, these methods by themselves allow for dense  
29 genome scans, but also identify thousands of markers, subsets of which can  
30 subsequently be genotyped in larger numbers of individuals using different genotyping  
31 technologies (Helyar *et al.* 2011).

32 The advent of next-generation sequencing technologies such as RAD sequencing is  
33 driving a shift from microsatellite to SNP genotyping in organisms with and without a

1 reference genome. The main advantages of SNPs are their high abundance and  
2 regular distribution across the genome, low scoring error rates, high reproducibility, a  
3 simple mutation model and the ability to concurrently screen neutral variation and  
4 regions of the genome under selection (Morin *et al.* 2004). Despite microsatellites  
5 typically presenting higher diversity per locus, a panel of several hundred SNPs is  
6 likely to be more informative than the 10-20 microsatellite loci used in standard  
7 population genetic studies (Helyar *et al.* 2011; Seeb *et al.* 2011), as shown in  
8 mapping (Ball *et al.* 2010), parentage (Hauser *et al.* 2011) and stock identification  
9 studies (Hess *et al.* 2011). The use of genotyping-by-sequencing methods to identify  
10 SNPs has many applications in ecological, evolutionary and population genetic studies.  
11 For example, Emerson *et al.* (2010) showed that RAD sequencing can be used to  
12 reveal previously unresolved genetic structure and detailed patterns of postglacial  
13 phylogeography of a non-model organism, the North American pitch planter mosquito,  
14 *Wyeomyia smithii*. Besides the assessment of population structure, genotyping-by-  
15 sequencing methods can also be used to detect signatures of selection and local  
16 adaptation. Hohenlohe *et al.* (2010) measured genome-wide genetic diversity across  
17 marine and freshwater populations of threespine stickleback (*Gasterosteus aculeatus*)  
18 using a high-density genome scan of 45,000 SNPs, which identified genomic regions  
19 exhibiting signatures of both balancing and directional selection.

20 Here, we use RAD tag sequencing to generate a resource of genome-wide SNPs in the  
21 European eel, *Anguilla anguilla*, a catadromous fish species with a particularly complex  
22 life cycle. After spawning in frontal zones of the southern Sargasso Sea, larvae cross  
23 the Atlantic Ocean following the Gulf Stream and metamorphose into glass eels upon  
24 reaching the Eastern Atlantic. Glass eels complete the migration into continental  
25 (freshwater, brackish, coastal) habitats as yellow eels, and after a highly variable  
26 feeding period, they metamorphose into silver eels that migrate back to the Sargasso  
27 Sea utilizing their high fat reserves, spawn once and die (van den Thillart *et al.* 2009).  
28 Remarkably, despite occupying a broad range of habitats from Subarctic environments  
29 in Iceland and northern Scandinavia to Subtropical environments in North Africa and  
30 the Mediterranean region, the European eel has been demonstrated to be a panmictic  
31 species (Als *et al.* 2011), a pattern that has also been revealed in the closely-related  
32 American eel *A. rostrata* (Coté *et al.* 2012).

1 In 2008, the long-term stock decline of the European eel prompted its inclusion in the  
2 IUCN (International Union for the Conservation of Nature) Red List of Threatened  
3 Species ([www.iucnredlist.org](http://www.iucnredlist.org)), with a current status as "critically endangered". All  
4 over Europe, the abundance of all life-stages of eel (glass eel, yellow eel, silver eel)  
5 has severely decreased since the mid 1980s. The recruitment of glass eels entering  
6 rivers has been exceptionally low over the last five years, with a decline of 99%  
7 (continental North Sea) and 95% (rest of Europe) in comparison with the 1960-1979  
8 levels (ICES 2011). Possible causes for the decline include anthropogenic factors such  
9 as overfishing, pollution, man-introduced parasites (the swimbladder nematode  
10 *Anguillicola crassus*) and diseases (EVEX virus) (van den Thillart *et al.* 2009), as well  
11 as climate and ocean current change (Knights 2003; Friedland *et al.* 2007;  
12 Bonhommeau *et al.* 2008).

13 A better understanding of crucial aspects of the biology of the European eel, including  
14 genetic diversity, effective population size and possible evolutionary responses to  
15 anthropogenic stressors, may promote measures to protect the species. Traditionally  
16 these issues have been addressed by using a low number of genetic markers due to  
17 the limited genomic resources available for eels. Two new rich sources of data have  
18 been recently made available: the first European eel transcriptome database Eeelbase  
19 (Coppe *et al.* 2010), which was recently updated to about 45,000 contigs (Pujolar *et al.*  
20 *et al.* 2012); and the first eel draft genome based on Illumina sequencing and a *de novo*  
21 assembly (Henkel *et al.* 2012), with the genome size determined to be 1.1 Gbp. The  
22 present study reports the generation of genomic RAD tags from a total of 30 glass  
23 eels from three separate sampling locations. The RAD tags enabled the discovery of  
24 novel candidate SNP markers, thereby providing the first genotyping-by-sequencing  
25 data set for a wide-spread, highly fecund marine fish species, and generating a SNP  
26 resource that can be used for selecting subsets of markers to be genotyped using  
27 medium- or high-throughput platforms.

28

## 29 **Material and Methods**

30

### 31 ***RAD tag sequencing***

32 Samples of glass eels were collected at three separate locations: one location in the  
33 western Mediterranean, the gulf of Valencia in Spain (39°49'N; 0°24'W), and two

1 locations in the eastern Atlantic, the Gironde estuary north of Bordeaux in France  
2 (45°15'N; 0°69'W) and the Burreishoole river in North-west Ireland (53°53'N; 9°34'W).  
3 Although the species is panmictic, sampling of geographically distinct localities  
4 accounts for the possibility that spatially and temporally variable selection might occur  
5 (Gagnaire *et al.* 2012). Genomic DNA was purified from a total of 30 individuals (10  
6 from each location) using standard phenol-chloroform extraction.  
7 Genomic DNA from each individual was digested with restriction enzyme EcoRI. A  
8 preliminary analysis suggested on average one cutting site every 2,346 bp. The  
9 digested product was ligated to a modified Illumina P1 adapter containing individual-  
10 specific nucleotide barcodes 4-8 bp long for sample tracking. All barcodes differed by  
11 at least two nucleotides to minimize sample mis-assignment due to sequencing error.  
12 Adapter-ligated fragments were subsequently pooled and sheared to an average size  
13 of 500 bp. Sheared DNA was separated by electrophoresis on a 2% agarose gel and  
14 fragments in the 350-500 bp size range were isolated using a MinElute Gel Extraction  
15 kit (Qiagen). After dsDNA ends were treated with end blunting enzymes and 3'-  
16 adenine overhangs were added, a modified Illumina P2 adapter was ligated. Finally,  
17 libraries were enriched by PCR amplification and RADs for each individual were  
18 sequenced (10 individuals per sequencing lane) on an Illumina Genome Analyzer II by  
19 Beijing Genomics Institute (BGI, Hong Kong, China) using paired-end reads.

20

### 21 ***RAD data analysis and SNP identification***

22 Sequence reads from the Illumina runs were sorted according to their unique barcode  
23 tag. Sequences were quality-filtered using the FASTX-Toolkit  
24 (<http://hannonlab.cshl.edu/fastx-toolkit>) and reads with ambiguous barcodes and of  
25 poor quality were removed from the analysis. A minimum Phred score of 10  
26 (equivalent to 90% probability of being correct) per nucleotide position was chosen,  
27 meaning that reads were dropped if a single nucleotide position had a score lower  
28 than 10. This is the Phred score generally used in SNP discovery studies (Ellison *et al.*  
29 2011; Scaglione *et al.* 2012; Van Bers *et al.* 2012; Wagner *et al.* 2012). Final read  
30 length was trimmed to 75 nucleotides, following a preliminary analysis that showed a  
31 substantial increase in the number of SNPs at the tails of the sequences (from position  
32 76 onwards), suggestive of sequencing errors (Figure 1). For subsequent analyses,  
33 only the first (left) paired-read was used. The DNA fragments created by RAD tag

1 library preparation have a restriction site at one end and are randomly sheared at the  
2 other end, which results in each instance of a restriction site sequence being sampled  
3 many times by the first reads and the genomic DNA sequence in the nearby region  
4 being randomly sampled at a lower coverage by the second paired-end reads (Etter *et*  
5 *al.* 2011), which are therefore less suitable for calling SNPs.

6 Sequence reads were aligned to the European eel genome draft  
7 ([www.eelgenome.com](http://www.eelgenome.com)) using the un-gapped aligner Bowtie version 0.12.8 (Langmead  
8 *et al.* 2009). A maximum of two mismatches between the individual reads and the  
9 genome were allowed and alignments were suppressed for a particular read when  
10 more than one reportable alignment existed, thereby decreasing the risk of  
11 paralogous sequences in the data.

12 The reference-aligned data were then used to assemble the RAD sequences into loci  
13 and identify alleles using the ref\_map.pl pipeline in Stacks version 0.9995 (Catchen *et*  
14 *al.* 2011). First, exactly-matching sequences are aligned together into stacks, which  
15 are in turn merged to form putative loci. At each locus, nucleotide positions are  
16 examined and SNPs are called using a maximum likelihood framework. Second, a  
17 catalog is created of all possible loci and alleles. Third, each individual is matched  
18 against the catalog. A minimum stack depth of 10 reads was used, which is the  
19 number of exactly matching reads that must be found to create a stack in an  
20 individual. Finally, the program Populations in Stacks was used to process all the SNP  
21 data across individuals. The minimum number of individuals to process a locus was  
22 set to 66.7% of the individuals sequenced.

23 Genome-wide measures of genetic diversity, including observed ( $H_o$ ) and expected  
24 ( $H_e$ ) heterozygosities and nucleotide diversity ( $\pi$ ), were calculated at each nucleotide  
25 site for all individuals as described in Hohenlohe *et al.* (2010). Using the average  
26 nucleotide diversity across all loci, long-term effective population size ( $N_e$ ) was  
27 estimated using  $\pi = 4 * N_e * \mu$  (Tajima 1983), where  $\mu$  is the mutation rate per site per  
28 generation. SNPs have relatively low mutation rates ( $1 \times 10^{-8}$  -  $1 \times 10^{-9}$  per generation;  
29 Brumfield *et al.* 2003) in comparison with other markers such as microsatellites that  
30 have mutation rates per generation of the order of  $10^{-4}$ .

31 Finally, batch BLAST similarity searches were conducted locally for all loci in the  
32 catalog using BLAST+ (NCBI). All sequences were blasted against the predicted  
33 complete transcripts from either scaffolds or unscaffolded contigs in the European eel

1 genome database ([www.eelgenome.com](http://www.eelgenome.com)). BLASTN searches were conducted using  
2 default parameters. Alignments with an e-value < 0.001 were considered significant.  
3 In case of multiple hits, best match was kept. Different annotation similarity cut-off  
4 values (60%, 80%, 90%) were considered.

5

## 6 **Results**

7 Sequencing of the RAD libraries generated an average of 8.67 million reads of 90 bp  
8 per individual, prior to any quality filtering. The number of reads ranged from 5.33 to  
9 13.03 million reads per individual. After quality filtering, on average 6.94 million  
10 (80.2%) sequences per individual were retained and 1.73 million (19.8%) sequences  
11 were eliminated. Retained sequences presented a mean quality score of 38.61, a  
12 median of 39.41 and a GC content of 40.6% (Table 1).

13 Out of the retained sequences, an average of 4.89 million (70.41%) aligned to the  
14 European eel draft genome, 1.75 million (25.17%) were not aligned and 306,969  
15 (4.42%) sequences were discarded due to alternative alignments (more than one  
16 reportable alignment existed) (Table 1).

17 Aligned sequences were assembled into an average of 489,870 stacks per individual  
18 and subsequently into a set of 328,812 loci. Using a minimum coverage of 10 reads  
19 per individual, an average of 202,923 (61.5%) loci were retained. Average coverage  
20 was  $22.52 \pm 2.18$  read per locus. A total of 125,890 (38.5%) loci were discarded per  
21 individual due to insufficient depth of coverage (Table 1). The ratio between observed  
22 and expected loci (based on the number of EcoRI cutting sites) was 65.1% when  
23 using a minimum stack depth of 10 reads per locus and 88.0% when using a  
24 minimum stack depth of 1 read per locus.

25 A catalog of 422,634 loci was constructed using all 30 individuals. After a final filtering  
26 step focused on loci genotyped in >20 out of the 30 individuals, a total of 142,509 loci  
27 were retained for SNP discovery. Out of these, 13,220 (9.27%) loci were  
28 monomorphic, 8,770 (6.14%) loci showed more than 2 alleles per individual (and  
29 were consequently eliminated from further analyses) and 120,539 (84.58%) were  
30 polymorphic, producing a total of 530,030 candidate SNP markers.

31 Average number of SNPs per locus was 3.96, ranging between 1 and 22 (Figure 2).  
32 Only 14.70% of the loci presented one single SNP, with 2 SNPs being the most  
33 frequent (17.61%). SNPs were evenly distributed across nucleotide positions in the



1 sequence reads and no apparent increase of SNPs toward the end of the reads was  
2 observed. About two thirds of the SNPs proved to be transitions in our dataset, with  
3 an observed transition:transversion ratio of 1.6:1 (Figure 3).

4 In order to support the validity of the large number of SNPs detected, data was re-  
5 analyzed using different parameters in the analysis. Firstly, we tested the effect of the  
6 alpha value used for the chi-square significance level when SNP calling. Similar results  
7 were obtained when using the default alpha of 0.05 (530,030 SNPs) or when using a  
8 more stringent alpha of 0.001 (527,352 SNPs), with a difference of less than 1%.

9 Secondly, we tested the effect of quality filtering using different Phred scores. Using a  
10 more conservative Phred score of 20, a large number of SNPs was still detected  
11 (461,380 SNPs). The use of different Phred scores had no apparent effect on the total  
12 number of loci (422,634 using a Phred score of 10; 407,401 using a Phred score of  
13 20), number of loci with more than two alleles (6.1% using a Phred score of 10; 5.8%  
14 using a Phred score of 20), average number of SNPs per locus (3.96 using a Phred  
15 score of 10; 3.89 using a Phred score of 20) and maximum number of SNPs per locus  
16 (over 20 in both cases). Thirdly, we re-analyzed all data using also the second (right)  
17 paired-end for alignment (but not for SNP calling), which makes the process more  
18 conservative. By comparing the results obtained when using the left paired-end only  
19 and when using both left and right paired-ends for alignment, we can determine if  
20 those loci presenting high numbers of SNPs are the consequence of poor alignment.

21 Using both paired-ends, loci with high number of SNPs were still detected, up to 21  
22 SNPs per loci, and the average number of SNPs per loci was 3.64, similar to the value  
23 found when using only the left paired-end (3.96). The fact that loci with over 20 SNPs  
24 were found independently of quality filtering, SNP calling or alignment procedure  
25 suggests that the method used for SNP discovery is accurate.

26 SNPs were widely distributed across the genome and were found in a total of 4,779  
27 different contigs and a total of 19,703 different scaffolds. When loci sequences were  
28 compared to the European eel genome using BLASTN, a significant similarity was  
29 found for 10,376 (6.8%) loci. Monomorphic loci showed a higher association with  
30 transcripts from either scaffolds or contigs in the eel genome (10.1%) than  
31 polymorphic loci (6.4%). Few loci were annotated, 0.2% using a cut off of 90, 0.3%  
32 using a cut off of 80 and 3.3% using a more relaxed cut off of 60% similarity.

33 Annotations were higher in monomorphic loci (0.2% using a cut off of 90, 1.1% using

1 a cut off of 80 and 5.7% using a cut off of 60) than in polymorphic loci (0.1% using a  
2 cut off of 90, 0.7% using a cut off of 80 and 3.1% using a cut off of 60).  
3 Finally, genome-wide measures of genetic diversity were calculated from the SNP  
4 data. A sequence length of 70 nucleotides was considered, since the first 5 nucleotides  
5 constitute the recognizing sequence motif for the restriction endonuclease. Substantial  
6 variation was identified, with average nucleotide diversity ( $\pi$ ) equal to  $0.00529 \pm$   
7  $0.00110$  across all 30 individuals included in the study. Results varied widely across  
8 loci, ranging from 0.00048 to 0.00737. Average observed and expected  
9 heterozygosity were 0.00468 and 0.00518, respectively.  
10 Using the average nucleotide diversity across all loci, long-term effective population  
11 size ( $N_e$ ) was estimated using Tajima's (1983) formula  $\pi = 4 * N_e * \mu$ , where  $\mu$  is the  
12 mutation rate per site per generation.  $N_e$  was estimated to range between 132,000  
13 (using a mutation rate of  $1 \times 10^{-8}$  per site per year) and 1,320,000 (using a mutation  
14 rate of  $1 \times 10^{-9}$  per site per year).  
15 As a final step, we generated a SNP resource available as an Excel spreadsheet (Table  
16 S1), including sequences of RAD tags, identified SNPs and their position in the  
17 European eel draft genome. For the resource, we excluded those loci in which all SNPs  
18 were singletons. In total the resource includes 82,425 loci in which at least one SNP  
19 was present in a minimum of two individuals. For these loci, apparent singleton SNPs  
20 are also reported since their presence may be relevant for primer design and for  
21 assessing if the SNPs are found in particularly variable genomic regions. The total  
22 number of SNPs in the resource is 376,918.

23

## 24 **Discussion**

### 25 **Large Scale SNP identification**

26 We report the discovery of a large number of SNPs in the European eel genome using  
27 the RAD sequencing approach. After excluding those loci in which all SNPs were  
28 singletons, we generated a large resource consisting of 82,426 loci and 376,918  
29 associated SNPs. While the amount of genomic resources available for this species are  
30 rapidly increasing, with the recent release of a draft genome, no genome-wide set of  
31 SNP markers was available until now. The generation of such a large panel of novel  
32 SNPs represents a major step in terms of genomic resources available for this species  
33 (Table S1). In this sense, only 49 microsatellite markers have been developed to date

1 in the European eel, including a panel of 12 dinucleotide microsatellites identified from  
2 enriched libraries (Wielgoss *et al.* 2008) and a larger set of 28 expressed sequence  
3 tag (EST)-linked microsatellite loci (Pujolar *et al.* 2008). Additionally, 232 proteins,  
4 177 ESTs and the complete mitochondrial genome are available in GenBank. The low  
5 number of markers available has somehow constrained genetic studies during the last  
6 two decades, and most studies have been conducted using <20 (or even <10)  
7 microsatellite loci. While classic population and conservation studies based on a few  
8 markers provide a “snapshot” of the variation in the genome, the panel of novel SNPs  
9 presented here will facilitate the development of population genomics studies on the  
10 European eel. Obviously, such studies can proceed using RAD sequencing for more  
11 samples, or they can make use of the generated SNP resource (Table S1) for selecting  
12 subsets of markers for genotyping in high numbers of individuals. The latter would be  
13 particularly advantageous when focusing on specific genes or parts of the genome or  
14 when analyzing degraded samples, such as DNA extracted from historical samples of  
15 otoliths or other hard parts (Nielsen & Hansen 2008), for which RAD sequencing and  
16 related methods are not suitable (Davey *et al.* 2011).

17 The feasibility of genome-scan approaches has been illustrated by several recent  
18 studies in a variety of organisms, including eukaryotes (Ellison *et al.* 2011), plants  
19 (Namroud *et al.* 2008; Turner *et al.* 2010), invertebrates (Turner *et al.* 2005, 2008)  
20 and fishes (Hohenlohe *et al.* 2010; Willing *et al.* 2010; Jones *et al.* 2012b). Genome-  
21 scan approaches such as SNP discovery using genotyping-by-sequencing can also  
22 provide a better understanding of adaptive evolution by means of identifying genes  
23 associated with ecologically important traits. Candidate genes and genomic regions  
24 can be identified using an  $F_{ST}$  outlier approach by detecting loci showing increased or  
25 decreased differentiation across populations compared to neutral expectations,  
26 suggestive of directional or purifying natural selection. Specifically for a panmictic  
27 species like the European eel, SNP based genome scans could be used to test within-  
28 cohort selection resulting from geographically varying environmental conditions  
29 encountered by glass eels across different regions of Europe and North Africa. In the  
30 case of the American eel, the recent study of Gagnaire *et al.* (2012) identified SNPs  
31 under possible temperature-related selection, with 13 loci showing correlations  
32 between allele frequencies and environmental variables across the entire species  
33 range. Moreover, introduced pathogens and parasites may have contributed to the

1 recent decline of the European eel (van den Thillart *et al.* 2009). Retrospective  
2 monitoring of SNPs associated with immune system related genes could be conducted  
3 based on contemporary and historical samples (e.g. archived otoliths) (Hansen *et al.*  
4 2012), which would allow for testing for possible adaptive responses to pathogens and  
5 parasites in the species.

6

### 7 **High SNP density points to large effective population size in the European eel**

8 One interesting result in our study is the high density of SNPs identified, with an  
9 average of 3.96 SNPs per locus and a maximum of 22. Sequencing errors, mostly  
10 found in the last nucleotide positions of the sequence reads, can mistakenly be  
11 identified as SNPs. If a substantial number of predicted SNPs in the dataset are the  
12 result of sequencing errors, an increase in the amount of SNPs toward the tails of the  
13 reads is expected. This was apparent in a pre-analysis of sequences trimmed to 80 bp  
14 showing a 20% over-representation of SNPs in positions 76-80. The fact that SNPs  
15 were equally distributed over the reads after trimming all sequences to 75 bp,  
16 indicates that the majority of our SNPS are not the result of sequencing errors and  
17 that our large scale SNP identification approach is valid. Additionally, we calculated  
18 the transition:transversion ratio of the SNPs in our dataset. If polymorphisms were  
19 introduced at random, a transition (A<->G or C<->T) to transversion (A<->C, A<-  
20 >T, C<->G, G<->T) rate of 1:2 would be expected. The SNPs in our dataset showed  
21 a transition:transversion ratio of 1.6:1, which suggests a very small influence of  
22 sequencing error on SNP calling. Similar transition:transversion ratios have been  
23 reported in the eggplant (1.65:1; Barchi *et al.* 2011) and the great tit (1.7:1; Van  
24 Bers *et al.* 2010). The fact that the number of SNPs found per locus did not change  
25 when applying more conservative quality filtering, SNP calling significance level and  
26 alignment procedures further supports the validity of the SNPs.

27 One explanation to the substantial polymorphism detected might be that the vast  
28 majority of our data is intergenic and intronic, as suggested by the low number of loci  
29 annotated using BLAST analysis (0.2-5.7% depending on the sequence similarity  
30 criterion used). In comparison, 2% of validated SNPs generated by deep sequencing  
31 of a reduced representation library were associated with rainbow trout transcripts  
32 (Castaño-Sanchez *et al.* 2009), and similar values have been found in humans (2%)  
33 and chimpanzee (1%) SNPs (Hodgkinson *et al.* 2009). Despite the high occurrence of

1 SNPs in our study, the presence of a large number of singletons and alleles in low  
2 frequency resulted in only a moderately high nucleotide diversity ( $\pi = 0.00529$ ).  $\pi$  also  
3 allows to estimate the long-term effective population size ( $N_e$ ) using  $\pi = 4 * N_e * \mu$  in a  
4 model in which sites evolve neutrally. Nevertheless, it should be noted that the model  
5 assumes an idealized population with random mating and constant size, which might  
6 not be necessarily met in the case of the European eel. The estimated  $N_e$  ranged  
7 between 132,000 and 1,320,000 individuals, depending on the mutation rate used, a  
8 much larger value than those previously reported in the literature. Using seven  
9 microsatellite loci, Wirth and Bernatchez (2003) estimated a long-term  $N_e$  of 4,410-  
10 5,388 individuals inferred by the coalescent-based genealogical method in MSVAR  
11 (Storz & Beaumont 2002). Using a larger panel of 22 EST-derived microsatellite loci,  
12 Pujolar *et al.* (2011) estimated a long-term  $N_e$  of 5,444-10,474 individuals inferred by  
13 a different Bayesian genealogy sampler (LAMARC; Kuhner 2006), which was  
14 consistent with the estimated values of short-term  $N_e$  of 5,031 (2,986-12,810)  
15 inferred by the comparison of allele frequencies across samples. The differences  
16 across studies, with a higher long-term  $N_e$  estimated in our study, can be due to the  
17 number and nature of microsatellite loci. In particular, MSVAR and LAMARC assume a  
18 simplistic stepwise mutation rate, whereas mutational properties at microsatellite loci  
19 are in reality more complex (Di Rienzo *et al.* 1994). The estimation of a relatively high  
20 effective population size is not surprising given that the species consists of one single  
21 large panmictic unit (Als *et al.* 2011). Nevertheless, it might be seen to contrast with  
22 the low abundance of recruitment and landings of yellow and silver eels occurring all  
23 over Europe (ICES 2011). However, it should be noted that this is a historical  $N_e$   
24 estimate, whereas a short-term  $N_e$  estimate would be required to detect the recent  
25 declines (Waples 2005).

26  
27 Collectively, the generation of a resource of 82,425 loci and 376,918 associated SNPs  
28 provides a valuable tool for future population genetics and genomics studies in the  
29 European eel and allows for targeting particularly interesting regions of the eel  
30 genome. All RAD tag sequences and associated SNPs are presented in a spreadsheet  
31 along with their map position in the draft eel genome (Table S1). Such resources were  
32 until recently only available for model organisms, whereas European eel must  
33 definitely be considered a non-model organism. Crucial aspects of its life cycle are still

1 unresolved and attempts to artificially propagate the species have so far proven  
2 unsuccessful (Tomkiewicz 2012). Hence, the generated eel SNP resource provides a  
3 clear illustration of the advances in next-generation sequencing and its potentials for  
4 overcoming the gap between model and non-model species.

5

## 6 **Acknowledgements**

7 We thank Annie Brandstrup for technical assistance, Russel Poole, Javier Lobon and  
8 Eric Feunteun for samples and Julian Catchen for advice on data analysis using  
9 Stacks. We also thank the subject editor and three anonymous referees for comments  
10 on a previous version of the paper. We acknowledge funding from the Danish Council  
11 for Independent Research, Natural Sciences (grant 09-072120).

12

## 13 **Author's contribution**

14 MMH and PFL conceived and designed the project. JMP, MMH and MWJ conducted all  
15 bioinformatics analyses. JBJ and LC were involved in data generation. JMP wrote the  
16 manuscript with contributions from MMH, MWJ, LZ, JF, TDA, PFL and GEM.

17

## 18 **Data Accessibility Statement**

19 Sequence reads have been deposited in the NCBI Sequence Read Archive (Accession  
20 number SRP020485).

21

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- 25

1 **Supporting Information**

2 **Table S1.** Spreadsheet encompassing all RAD tag sequences and associated SNPs,  
3 along with their position in the draft eel genome.

4

5

1 **Figure 1.** Number of SNPs per nucleotide position (1-80). There is an apparent  
2 increase in number of SNPs in the last 5 nucleotides (76-80), suggestive of  
3 sequencing errors, which were consequently removed from the analyses.

4

5 **Figure 2.** Distribution of the number of SNPs per loci.

6

7 **Figure 3.** Transitions and transversions occurring within a set of 551,429 European  
8 eel SNPs.

9

10

1 **Table 1.** Statistics describing the distribution of different properties of RAD sequences  
 2 after each step of filtering (FASTX-Toolkit), alignment to the eel draft genome  
 3 (Bowtie) and assemblage into loci (Ref\_map.pl).

4  
5

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<b>FASTX-Toolkit</b>										
<b>Raw reads</b>	<b>Filtered reads</b>	<b>% Eliminated</b>	<b>Mean Q</b>	<b>Q1</b>	<b>Med</b>	<b>Q3</b>	<b>%A</b>	<b>%C</b>	<b>%G</b>	<b>%T</b>
8670526	6942282	19.8	38.6	38	39.4	40	29.8	20.5	20.1	29.7

---

<b>Bowtie</b>						
<b>Reads</b>	<b>Aligned</b>	<b>% Aligned</b>	<b>Non-aligned</b>	<b>% Non-aligned</b>	<b>Discarded</b>	<b>% Discarded</b>
6942282	4886517	70.4	1749063	25.2	306969	4.4

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<b>Ref_map.pl</b>						
<b>Reads</b>	<b>Stacks</b>	<b>Loci</b>	<b>Loci used</b>	<b>% Loci used</b>	<b>Loci discarded</b>	<b>% Loci discarded</b>
4886517	489870	328812	202923	61.5	125889	38.5

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6  
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