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Natural selection influences AFLP intraspecific genetic variability and introgression patterns in Atlantic eels

P. A. GAGNAIRE,* V. ALBERT,† B. JÓNSSON‡ and L. BERNATCHEZ†
**Institut des Sciences de l'Evolution (ISEM UMR 5554 CNRS-UMII), Université de Montpellier II, CC 065, Place E. Bataillon, 34095 Montpellier cedex 5, France, †Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, Canada G1V 0A6, ‡Institute of Freshwater Fisheries, Northern Division, Sauðárkrókur, Iceland, IS 550*

Abstract

Investigating patterns of genetic variation in hybridizing species provides an opportunity to understand the impact of natural selection on intraspecific genetic variability and interspecific gene exchange. The Atlantic eels *Anguilla rostrata* and *A. anguilla* each occupy a large heterogeneous habitat upon which natural selection could differentially shape genetic variation. They also produce viable hybrids only found in Iceland. However, the possible footprint of natural selection on patterns of genetic variation within species and introgressive hybridization in Icelandic eels has never been assessed. We revisited amplified fragment length polymorphism data collected previously using population genomics and admixture analyses to test if (i) genetic variation could be influenced by non-neutral mechanisms at both the intra- and interspecific levels, and if (ii) selection could shape the spatio-temporal distribution of Icelandic hybrids. We first found candidate loci for directional selection within both species. Spatial distributions of allelic frequencies displayed by some of these loci were possibly related with the geographical patterns of life-history traits in *A. rostrata*, and could have been shaped by natural selection associated with an environmental gradient along European coasts in *A. anguilla*. Second, we identified outlier loci at the interspecific level. Non-neutral introgression was strongly suggested for some of these loci. We detected a locus at which typical *A. rostrata* allele hardly crossed the species genetic barrier, whereas three other loci showed accelerated patterns of introgression into *A. anguilla* in Iceland. Moreover, the level of introgression at these three loci increased from the glass eel to the yellow eel stage, supporting the hypothesis that differential survival of admixed genotypes partly explains the spatio-temporal pattern of hybrid abundance previously documented in Iceland.

Keywords: AFLP, *Anguilla*, introgressive hybridization, natural selection, outlier loci, species genetic barrier

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Introduction

Discerning the relative influence of neutral vs. selective processes acting in natural populations is a fundamental step towards the comprehension of species' evolution. Populations living in heterogeneous habitats are likely to undergo diverse selective constraints that can differentially shape genetic variation among them, occasionally leading to reproductive isolation (Mayr 1947). Direct observation of the genetic effects of natural selection can be conveniently assessed when adaptive phenotypic traits and genes underlying these adaptations are known. In most nonmodel organisms, a priori knowledge concerning the genes governing adaptive traits is often not available, thus, it becomes necessary to use indirect methods to identify loci potentially under selection. Such 'population genomics' approaches are based on the principle of screening sufficient number of molecular markers randomly distributed across the whole genome in a large number of individuals, to detect loci whose level of differentiation between populations exceeds that expected under neutral expectations.

Correspondence: Louis Bernatchez, Fax: 1 418 656 7176;
E-mail: louis.bernatchez@bio.ulaval.ca

These 'outlier loci' are assumed to be located in the vicinity of actual genes under selection due to genetic hitchhiking (Beaumont & Nichols 1996; Luikart *et al.* 2003; Beaumont & Balding 2004; Beaumont 2005; Stinchcombe & Hoekstra 2007; Via & West 2008). Ideally, outlier identification in a genome scan needs to be followed up with approaches such as bacterial artificial chromosome (BAC) library screening to identify functionally important polymorphic sites (e.g. Wilding *et al.* 2001; Wood *et al.* 2008). Nevertheless, such an approach provides an efficient means to estimate the proportion of loci involved in adaptive divergence, and compare sets of outlier loci identified in various selective contexts (e.g. Wilding *et al.* 2001; Campbell & Bernatchez 2004; Rogers & Bernatchez 2007). For instance, population genomics allowed specifying the role of environmental factors in shaping the distribution of genetic diversity along environmental gradients (Bonin *et al.* 2006; Jump *et al.* 2006). In addition, the analysis of differentiation patterns among the genomes of closely related species suggests that divergent selection usually concerns a few loci at which gene flow is dramatically reduced, whereas most of the genome appears permeable to gene exchange (Scotti-Saintagne *et al.* 2004; Savolainen *et al.* 2006; Minder & Widmer 2008).

Population genomics has also improved our understanding of the architecture of genetic barriers between hybridizing species. Mosaic genomes reveal delays, or even barriers to introgression at loci undergoing negative selection in hybrids and in the recipient species (Martinsen *et al.* 2001). Such effects can be due to disruption of epistatic interactions between co-adapted genes by recombination and may be largely responsible for hybrids inferiority (Burke & Arnold 2001). Conversely, hybridization may also accelerate the rate of introgression at positively selected loci (Kim & Rieseberg 1999; Martin *et al.* 2006; Whitney *et al.* 2006). Therefore, hybridization can have a variety of effects in species evolution, and contrasted footprints are expected to be found at the genome level between neutral, negatively and positively selected genes. A genome-scan approach is appropriate to observe these differential patterns of introgression and help to understand the particular architecture of the barrier to gene flow between hybridizing species (Rieseberg *et al.* 1999; Rogers *et al.* 2001).

The present study aimed at testing the influence of natural selection on genetic variation at various scales in Atlantic eels. The two Atlantic eel species *Anguilla rostrata* and *A. anguilla* each occupies a large heterogeneous habitat, corresponding to most of North America and Europe (including Iceland and Northern Africa), respectively. It is widely accepted that each of the two Atlantic eel species has a unique spawning area, both located and overlapping in the Sargasso Sea (Schmidt 1925; McCleave *et al.* 1987; see Appendix S1, Supporting Information). This aspect of eel life history led to the prediction that genes should be randomly exchanged within each species, which has been supported by a number of studies (e.g. De Ligny & Pantelouris 1973; Comparini *et al.* 1977; Avise *et al.* 1986; Lintas *et al.* 1998). However, the use of microsatellite markers later revealed weak albeit significant genetic differentiation in the European eel (Wirth & Bernatchez 2001; Daemen *et al.* 2001). Genetic patterns corresponding to isolation by distance (Wirth & Bernatchez 2001; Maes & Volckaert 2002) and isolation by time (Dannewitz *et al.* 2005; Maes *et al.* 2006; Pujolar *et al.* 2006) were also documented, therefore contradicting the results of earlier studies. In both species, a latitudinal cline of allozymic variation potentially induced by natural selection was also reported (Williams *et al.* 1973; Koehn & Williams 1978; Maes & Volckaert 2002). Therefore, the potentially confounding effects of natural selection on species genetic diversity must be investigated in greater detail to understand the nature of population structure in Atlantic eels.

Molecular analyses also recently showed that both eel species interbreed to produce viable hybrids that are only encountered in Iceland (Avise *et al.* 1990; Albert *et al.* 2006). Albert *et al.* (2006) further revealed that F_1 hybrids can successfully migrate back to the Sargasso Sea and reproduce. Passive larval transport through the Gulf Stream combined with heterogeneity of larval duration were proposed to explain why hybrids are geographically limited to Iceland (Albert *et al.* 2006; Kettle & Haines 2006). Furthermore, a puzzling spatio-temporal pattern emerged from the proportions of hybrids observed in Icelandic rivers. Albert *et al.* (2006) found higher first- and later-generation hybrid proportions in yellow eels compared to glass eels, and a latitudinal increase towards the north in the proportion of hybrids. However, the possibility of a temporal decrease in hybridization rate hampered tests of the hypothesis of differential survival between purebreds and hybrids in Iceland. Also, identification of loci potentially under the effect of selection was out of the scope of Albert *et al.*'s 2006 study. Therefore, the influence of natural selection on hybrid and pure European eel abundance in Iceland remains untested. Here, our main objective was to use population genomics and admixture statistical methods to revisit the amplified fragment length polymorphism (AFLP) data of Albert *et al.* (2006), in order to test if (i) intraspecific genetic structure of each species and if (ii) hybrid proportions and genetic composition found in Icelandic rivers were influenced by natural selection.

Materials and methods

Study species, sampling sites

Five developmental stages characterize the complex life cycle of Atlantic eels (Tesch 2003). The oceanic pelagic larvae, which undergo a several month drift through the

Table 1 Sampling location, abbreviation, date and size of each sample. Sampling year and life stage (G, glass eel; Y, yellow eel) were only provided when all individuals were homogeneous for these characteristics. Number of individuals in each of the six categories defined with NewHybrids was determined and detailed for each sample: Aro (parental *A. rostrata*), Aan (parental *A. anguilla*), F_1 (Aro × Aan), F_2 ($F_1 \times F_1$), BC_{Aro} (Aro × F_1) and BC_{Aan} (Aan × F_1)

					NewHybrids' categories					
Sampling location	Sample abbreviation	Sampling date	Sampling size	Life stage	Aro	F_1	F_2	BC_{Aro}	BC_{Aan}	Aan
North America										
Medomak River	ME	1999	45	Y	45	—	—	—	—	—
Boston Harbor	BO	1999	50	G	50	—	—	—	—	—
Wye River	WY	1999	48	Y	48	—	—	—	—	—
St Johns River	SJ	1999	50	Y	50	—	—	—	—	—
Total America	Aro	1999	193		193	—	—	—	—	—
Europe										
Elbe River	EL	1999	49	Y	—	—	—	—	—	49
Grand Lieu Lake	GL	1999	49	G	—	—	—	—	—	49
Minho River	MI	1999	45	G	—	—	—	—	—	45
Moulouya Oued	MO	1999	43	G	—	—	—	—	—	43
Total Europe	Aan	1999	186		—	—	—	—	—	186
Iceland										
Saudárkrókur	SA	2003	6	Y	—	4	—	—	2	—
Vatnsdalur	VA	2000	18	Y	—	1	2	—	2	13
Reykhólar	RE	2001	13	Y	—	5	1	—	—	7
Bár	BA	2003	49	Y	—	4	—	—	2	43
Vogslækur	VO	2000	50	G	—	8	—	—	5	37
		2001	50	G	—	3	1	—	2	44
		2002	49	G	—	1	—	—	—	48
		2003	49	G	—	3	—	—	2	44
		2001	36	Y	—	2	1	—	3	30
Seljar	SE	2001	48	G	—	4	2	—	4	38
		2001	46	Y	—	11	1	—	7	27
Vífilsstadavatn	VI	2001	50	G	—	1	2	—	6	41
		2002	45	Y	—	4	—	—	6	35
Grafarvogur	GR	2003	45	Y	—	1	—	—	2	42
Stokkseyri	ST	2001	46	G	—	—	2	—	1	43
		2003	50	G	—	—	—	—	1	49
		2003	49	Y	—	5	1	—	1	42
Öxnalækur	OX	2003	49	Y	—	1	—	—	4	44
Total Iceland			748		—	58	13	—	50	627
Iceland *A. anguilla* sample	IC	2001	57	Y	—	—	—	—	—	57
Iceland F_1 sample (70%)	F_1		33		—	33	—	—	—	—
Iceland F_2 sample (50%)	F_2		11		—	—	11	—	—	
Iceland BC_{Aan} sample (70%)	BC_{Aan}		38		—	—	—	—	38	—

North Atlantic Ocean, are called leptocephali. Larvae metamorphose into glass eels when they reach the continental shelves, and then temporarily settle in estuaries where they become pigmented. This corresponds to the transient elver stage that precedes the yellow eel phase. After 3–20 years spent in their growing continental habitat, yellow eels metamorphose into sexually mature silver eels and migrate back to the Sargasso Sea to reproduce. The present study focuses on the glass eel and the yellow eel stages.

Table 1 provides information concerning sampling location and date, sample size, life stage and individual hybrid status of the 1127 eels analysed with 373 AFLP loci by Albert *et al*. (2006) (see also Appendix S1, for a map showing the geographical distribution of sampling sites). In order to perform pairwise comparisons with *Anguilla anguilla* samples from the European continent, we built an Icelandic sample by pooling pure European eel individuals from the yellow eel samples of Seljar and Vogslækur, both collected in 2001 (called IC in Table 1 and Appendix S1). These two samples were chosen because they were temporally close to the European samples collected in 1999 and not geographically distant in Iceland.

Hybrid category assignment

We used NewHybrids software, which implements a multilocus allele-frequency model-based method for determining hybrid status (Anderson & Thompson 2002). This method performs individual clustering without any a priori knowledge of parental allele frequencies, and has the advantage of specifically assuming a mixture of parental and various hybrid classes in its probability model. Six categories corresponding to parental (pure American and pure European), F_1, F_2 and backcrosses (with BC_{Aro} individuals generated by crossing events between an F_1 and a pure *Anguilla rostrata* and BC_{Aan} individuals between an F_1 a pure *A. anguilla*) were considered. Individual posterior probabilities to belong to each hybrid category were estimated by Markov chain Monte Carlo method in a Bayesian framework. We initially set a posterior probability threshold of 0.7, above which individuals were assigned. This threshold was subsequently lowered to 0.5 for the F_2 category. Calculations were run using Jeffreys-type priors and a burn-in period of 50 000 iterations followed by 50 000 sweeps for sampling from the posterior distribution.

Outlier loci detection

We used the Dfdist program implementing the hierarchical-Bayesian approach of Beaumont & Balding (2004) to detect outlier loci. Null allele frequencies were first estimated at each locus of the empirical AFLP data set using Zhivotovsky's (1999) Bayesian approach , enabling F_{ST} values to be estimated for each locus (Weir & Cockerham 1984). A mean 'neutral' F_{ST} value supposedly uninfluenced by selected loci was then calculated after removal of 30% of the highest and 30% of the lowest F_{ST} values found in the empirical data set (see Bonin *et al*. 2006; Miller *et al*. 2007; Nosil *et al*. 2008). This 'trimmed' F_{ST} value was used to target the mean F_{ST} of 50 000 loci generated by coalescent simulation. Therefore, the F_{ST} distribution of these simulated loci was expected to be close to that of the neutral empirical data set. The outlier threshold was defined by an envelope delimited by the 0.005 and 0.995 quantiles of simulated F_{ST}. However, because the power to detect footprints of balancing selection is generally low (Beaumont & Nichols 1996; Beaumont & Balding 2004), only outliers that were candidates for directional selection were considered.

We searched for directional selection footprints at the intraspecific levels in North American and European locations, together with Iceland, by comparing all possible pairs of samples in each species. As some trimmed F_{ST} values were less than 0.005 or even slightly negative, they suggested that neutral F_{ST} was close to zero between some eel samples of the same species. Therefore, in order to perform locus simulations with Dfdist, a small positive F_{ST} of 0.005 was used (Miller *et al*. 2007). In this way, detection of outlier loci was more conservative. Moreover, pairwise analyses allow the identification of loci that are outliers in multiple pairs of populations. We considered loci that were detected as outliers in more than two pairwise comparisons as the most likely candidates, thus reducing type I error (Nosil *et al*. 2008). We then conducted an interspecific analysis by pooling pure individuals from the same species into an *A. rostrata* (Aro) and an *A. anguilla* (Aan) sample. Individuals from Iceland were not included in this analysis to avoid the influence of putative Iceland-specific selection.

Locus-specific introgression level

In order to detect the potential effects of natural selection in Icelandic hybrids, we tested departure from theoretical frequencies at each locus in each hybrid category. Because the determination of hybrid status with NewHybrids showed that only pure individuals occurred in continental locations of Europe (*A. anguilla*) and America (*A. rostrata*) (Table 1, see also Albert *et al*. 2006), we used the pooled *A. rostrata* (Aro) and *A. anguilla* (Aan) samples to estimate parental allele frequencies. At each locus in each species, we assumed Hardy–Weinberg equilibrium to estimate the frequency of the null allele from the square root of the null homozygote frequency. Using these observed parental frequencies, expected allele frequencies were then calculated for each of the three hybrid categories found in Iceland: F_1, F_2 and BC_{Aan} (see Results). A binomial test was then performed to test for significant deviation between observed and expected frequencies of band presence at each locus in each hybrid category. A Bonferroni correction was applied to avoid false positive detection due to type I errors.

Maximum-likelihood estimates of hybrid indices

We also applied a complementary approach based on the estimate of hybrid indices over nondiagnostic loci (Rieseberg *et al*. 1998, 1999). This allows the detection of candidate loci for which the introgression pattern has possibly been influenced by selection (Rogers *et al*. 2001). The model assumed two separated parental populations that both contributed to an admixed population. *Hybrid index for an individual* is an estimate of the proportion of its ancestors that belonged to each parental species at the generation before the first interbreeding event in its ancestry. Therefore, the joint estimate of hybrid index over all individuals reflects the relative contribution of each parental source to the admixed population based on individuals. Hybrid indices were estimated from allele frequencies of both parental samples assessed with the square root

Table 2 Number of polymorphic loci and outlier loci found in each intraspecific pairwise comparison for both Atlantic eel species. For each sample pair, the mean value and the range of outlier F_{ST}, the trimmed F_{ST} value and the presumed neutral F_{ST} value found with microsatellite markers (Wirth & Bernatchez 2003) are provided, along with the latitudinal distance separating localities. Sample abbreviations are as indicated in Table 1

Sample pair	No. of polymorphic loci	No. of outlier loci	Mean outlier F_{ST} value (min F_{ST} – max F_{ST})	Trimmed F_{ST}	Neutral F_{ST}	Latitudinal distance (degree)
Anguilla rostrata						
ME vs. BO	311	5	0.2089 (0.1646–0.2584)	0.0061	–0.0005	1.75
ME vs. WY	304	8	0.1978 (0.0920–0.3068)	0.0018	0.0034	5.20
ME vs. SJ	299	5	0.1504 (0.0812–0.2584)	–0.0004	0.0005	14.18
BO vs. WY	295	5	0.2777 (0.0678–0.4086)	0.0011	–0.0008	3.45
BO vs. SJ	304	3	0.2418 (0.1752–0.2888)	0.0067	–0.0016	12.43
WY vs. SJ	301	8	0.1627 (0.0679–0.2867)	0.0015	0.0037	8.98
Anguilla anguilla						
IC vs. EL	291	13	0.3381 (0.1423–0.6496)	0.0102	0.0016	9.70
IC vs. GL	291	9	0.2537 (0.1399–0.4388)	0.0110	–0.0009	17.48
IC vs. MI	293	12	0.2460 (0.0902–0.4344)	0.0018	–0.0008	22.70
IC vs. MO	303	7	0.2747 (0.1557–0.5605)	0.0054	0.0011	29.40
EL vs. GL	300	4	0.1767 (0.1355–0.2685)	0.0030	0.0003	7.78
EL vs. MI	305	9	0.2127 (0.0912–0.2773)	0.0032	0.0016	13.00
EL vs. MO	311	7	0.2813 (0.1208–0.4405)	0.0058	0.0017	19.70
GL vs. MI	294	2	0.1863 (0.1658–0.2068)	–0.0012	0.0005	5.22
GL vs. MO	295	4	0.1480 (0.1116–0.1700)	–0.0003	0.0007	11.92
MI vs. MO	300	0	—	–0.0041	0.0024	6.70

procedure. Likelihood functions were constructed following the method described in Rogers *et al*. (2001), and hybrid indices as well as their support (two log-likelihood units) were determined under the likelihood framework using R software (R Development Core Team 2004). The joint estimate of hybrid index over all Icelandic individuals was then used to calculate theoretical allele frequencies in the introgressed population of Iceland. For each locus, the deviation of observed frequencies from theoretical expectations was used to detect the possible influence of directional selection.

Correlation tests

Because the pattern of an AFLP locus consists of binary data, we used binomial logistic regression to test correlations between genotypes and other explanatory factors. We tested for the influence of the categorical factor 'life stage' (glass eel or yellow eel stage) and the continuous factors 'body length' and 'sample latitude' using R (R Development Core Team 2004). Yellow eel body length was considered as a rough surrogate for individual age.

Results

Intraspecific outlier detection

Trimmed F_{ST} values were close to zero in each of the six pairwise comparisons performed between *Anguilla rostrata* samples (Table 2). Outliers were detected in each comparison, and showed pairwise F_{ST} values ranging from 0.068 to 0.409 whereas neutral loci F_{ST} ranged between –0.038 and 0.211 (Fig. 1). A total of 22 out of 325 polymorphic loci were outliers in at least one comparison, five of them were detected in two pairs of samples and three were involved in more than two pairwise comparisons. Among these, loci L65 and L260 were detected as outliers in all comparisons involving the Wye River and Medomak River samples, respectively, whereas locus L179 was the only outlier to appear in comparisons between pairs that did not involve a sample in common (Fig. 1).

Low pairwise trimmed F_{ST} values calculated between *A. anguilla* samples revealed very weak neutral differentiation throughout the European eel distribution range (Table 2). Outliers were detected in every pairwise comparison except between Minho River and Moulouya Oued (Fig. 2). F_{ST} values at outlying loci ranged from 0.090 to 0.650, whereas neutral loci showed F_{ST} values ranging from –0.038 to 0.277. Nine out of the 334 polymorphic loci were outliers in one of the 10 pairwise comparisons performed, whereas seven were detected in two different pairs (Fig. 2). Among the six loci that were associated with a particular sample in more than two comparisons, five were associated with Iceland (L144, L278, L306, L337 and L372) and one with Elbe station (L22). Five other loci involved in more than two comparisons also appeared in independent sample pairs (L11, L17, L32, L69 and L368) (Fig. 2).

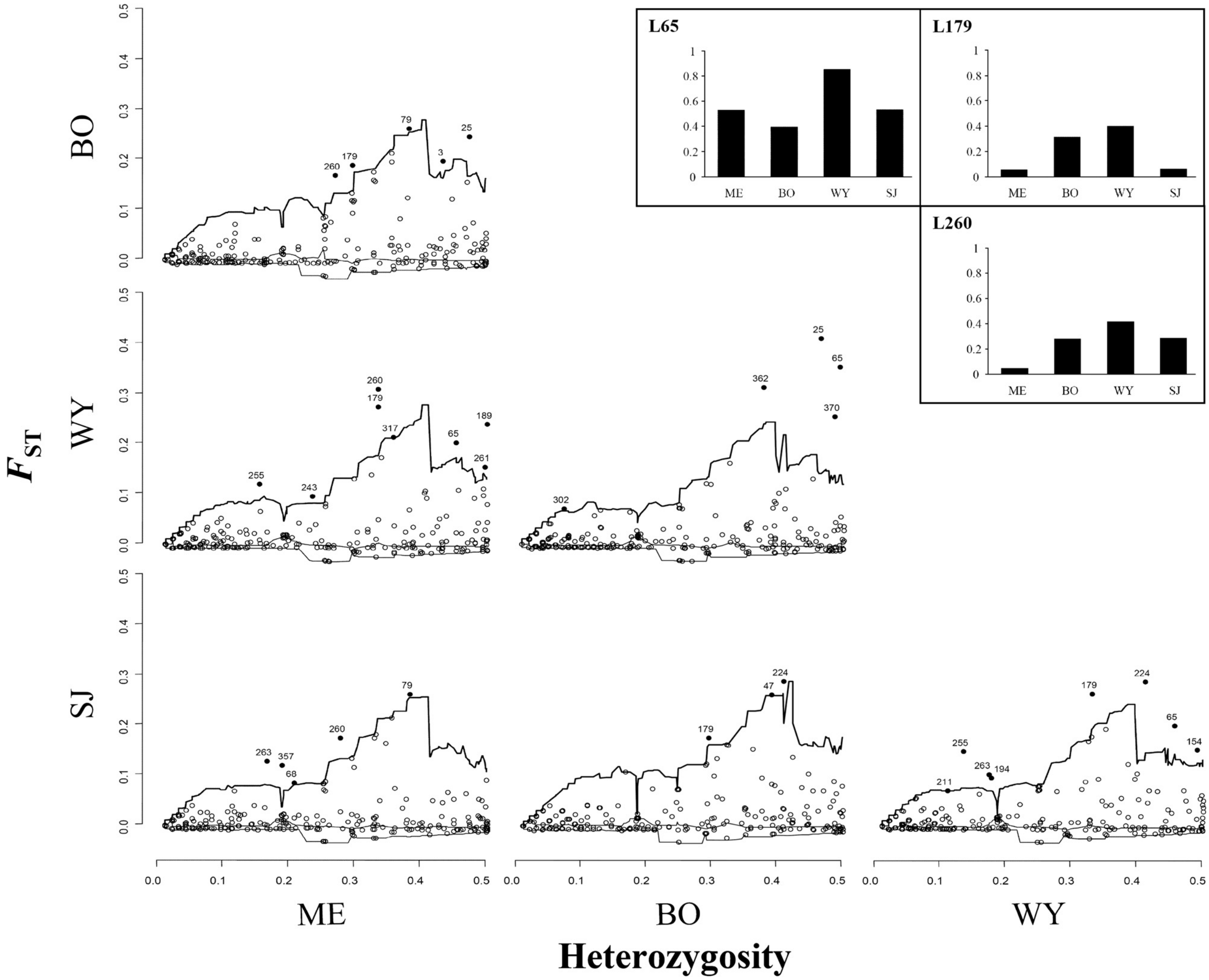

Fig. 1 Results of Dfdist analyses for *Anguilla rostrata* intraspecific comparisons. F_{ST} vs. heterozygosity plot is provided for each pairwise combination of samples. Open circles represent neutral loci falling below the 0.995 quantile's broad line, whereas candidate loci under divergent selection are represented by black circles accompanied by the locus number. Lower and middle lines in each plot, respectively, represent the 0.005 quantile and mean F_{ST} value over the range of heterozygosity. Frames at the upper right contain barplots showing dominant allele frequency in each locality for the three outlier loci detected in more than two sample pairs.

A significant positive correlation was found between latitudinal distance separating European localities and the number of outliers in the corresponding pairwise comparisons (Spearman's $\rho = 0.57$, $P = 0.04$). This correlation was more obvious when Iceland was excluded (Spearman's $\rho = 0.90$, $P = 0.01$). Moreover, there was a significant positive correlation between trimmed F_{ST} values and the number of outliers found in pairwise comparisons between *A. anguilla* localities (Spearman's $\rho = 0.72$, $P = 0.009$), which was also more obvious after withdrawing Iceland (Spearman's *rho* = 0.93, $P = 0.004$). No such correlation could be found when applying these tests in *A. rostrata*. No outlier among the 22 found between *A. rostrata* samples and the 27 between *A. anguilla* samples was detected as intraspecific outlier in both species.

Interspecific outlier detection

The interspecific comparison had a trimmed F_{ST} value of 0.0685. A total of 27 out of 321 (8.4%) polymorphic loci were identified as outliers in this analysis (Fig. 3). Three were also identified as *A. rostrata* intraspecific outliers (L243, L263 and L370) and four as intraspecific outliers in *A. anguilla* (L6, L278, L368 and L372). In this last case, Iceland was always involved in the intraspecific comparisons in which these outliers were detected.

Locus specific introgression level

The determination of individual hybrid status with NewHybrids revealed the occurrence of 33 F_1, 8 F_2, and 38

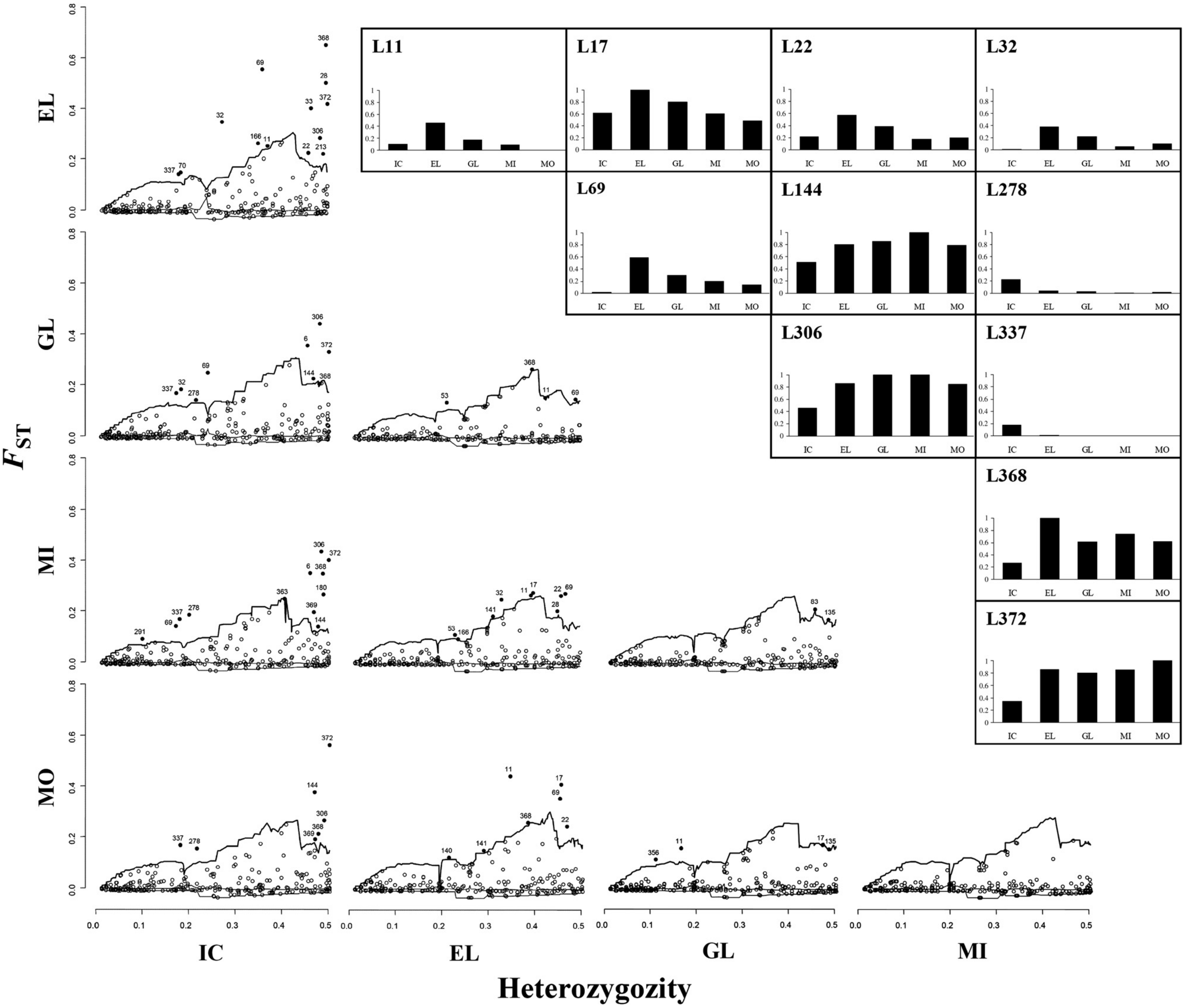

Fig. 2 Results of Dfdist analyses for *Anguilla anguilla* intraspecific comparisons. Plots were constructed as in Fig. 1, for every pairwise comparison in the European eel. Frames at the upper right contain barplots showing dominant allele frequency in each locality for the 11 outlier loci detected in at least three sample pairs.

BC_{Aan} individuals with a posterior probability greater than 70% (Table 1). At the 50% posterior probability level, 11 individuals were identified as F_2 hybrids. No BC_{Aro} individual was found in the samples. Results of binomial tests between allele frequencies estimated in each of the three hybrid categories detected, and the corresponding theoretical frequencies calculated from parental sources, are shown in Fig. 4a for F_1, 4b for F_2 and 4c for BC_{Aan} hybrid categories. Seven loci showed cumulatively a bias towards *A. rostrata* parental frequencies in at least one hybrid category and an outlying behaviour in the interspecific comparison. Among them, loci L6, L368 and L372 were also outliers in pure *A. anguilla* intraspecific comparisons involving Iceland. On the other hand, the interspecific outlier locus L236 showed a strong bias towards *A. anguilla* parental frequencies in each hybrid category.

Maximum-likelihood estimates of hybrid indices

After removal of loci that showed less than 1% frequency difference between *A. rostrata* and *A. anguilla* pooled samples, 303 loci out of 373 were retained to estimate hybrid indices. Differences between dominant (presence of AFLP band) allele frequencies of parental samples ranged from 1 to 98%. Based on these loci, the joint maximum-likelihood estimate of hybrid index over all Icelandic individuals was 0.135 (two units of support: 0.129–0.140). This reconfirmed that individual genetic composition most likely originated

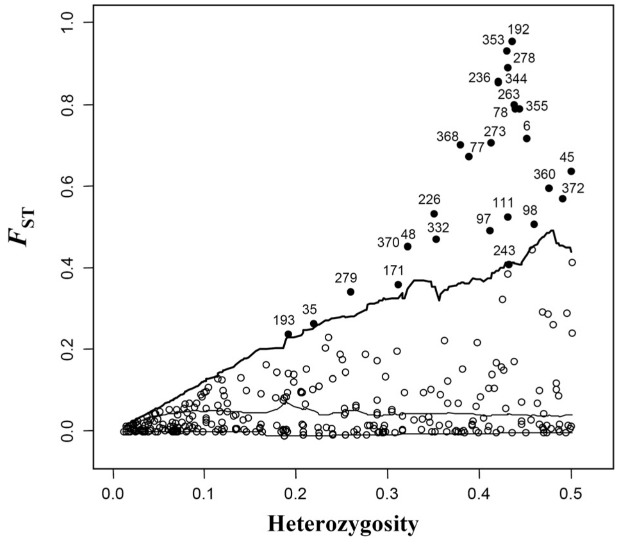

Fig. 3 Results of Dfdist analysis for interspecific comparison, showing outlier candidate loci for divergent selection between the two Atlantic eel species.

from *A. anguilla*, but that Icelandic eels were introgressed by *A. rostrata*, as reported by Albert *et al.* (2006). The distribution of locus frequency deviation from theoretical expectations using this 0.135 joint hybrid index is presented in Fig. 5. With the exception of locus L192, all loci that significantly deviated from theoretical frequencies in at least one hybrid category (Fig. 4) showed the same deviation direction with the hybrid index approach (Fig. 5). Setting a 95% threshold to detect loci that departed the most from neutral expectations at both extremities of the distribution allowed us to refine the identification of markers potentially under selection. Five loci showed an overrepresentation of European alleles, and among these, three were detected to significantly deviate from theoretical frequencies in at

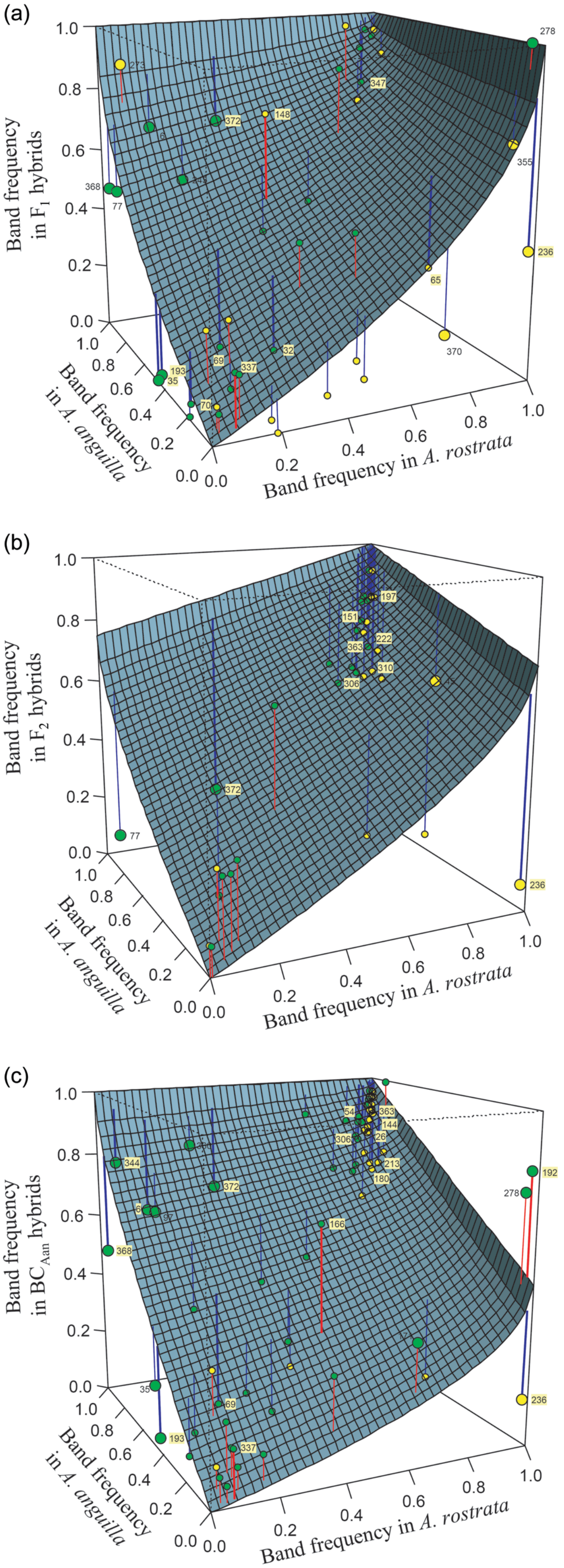

Fig. 4 Three-dimensional plots showing the results of locus deviation tests in hybrid categories. In each plot, the grey-coloured squared surface shows the theoretical probability of observing the dominant marker as a function of band presence frequency in each parental species. For each locus, the difference between observed and theoretical frequency of band presence was represented (only if significant) by a vertical line joining the locus coordinates to its projection on the theoretical surface. Vertical lines were red-coloured when localized above the surface and blue-coloured when localized under. Loci were represented by points whose size indicated if they were interspecific outliers (large points accompanied by locus number) or not (small points). The colour of a point was yellow when band frequency was biased towards *Anguilla anguilla* parental frequency and green when biased towards *A. rostrata*. Loci whose deviation was still significant after Bonferroni correction had a broader deviation line and their identifying number was labelled with a light brown tag. Results are provided in separate plots for each hybrid category obtained with NewHybrids: F_1 (4a), F_2 (4b) and BC_{Aan} (4c).

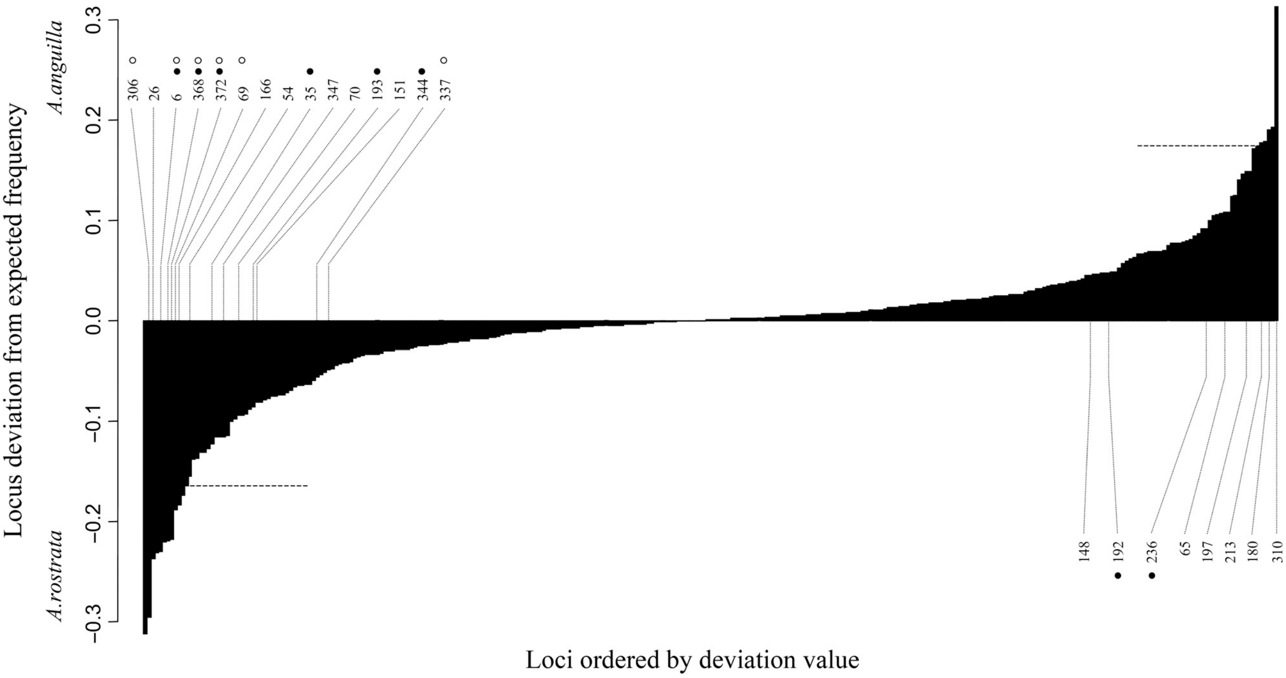

Fig. 5 Deviation of observed marker frequencies from theoretical expectations given the 0.135 estimated hybrid index over all individuals, for the whole Icelandic sample using polymorphic loci. Deviation values are represented by black histograms ordered from loci characterized by an allelic composition of *Anguilla rostrata* (negative deviation) to *A. anguilla* origin (positive deviation). Loci that showed significant deviation after Bonferroni correction in at least one hybrid category (Fig. 4) were labelled with their identification number. This number is followed by a black circle if the locus was also an interspecific outlier, and/or an open circle if the locus was also an intraspecific outlier in at least two pairwise comparisons involving Iceland. The two horizontal lines delimit loci whose absolute deviation value exceeded the one-sided 95% confidence interval for the distribution of loci deviation's absolute value.

least one hybrid category. The locus L236, which showed highly reduced introgression pattern in each hybrid category, was in the *A. anguilla* part of the distribution, but its deviation value did not exceed the 95% threshold. Eleven loci showing allele frequencies biased towards the American eel composition were outside the one-sided 95% confidence interval. Among these, five markers were already detected in deviation tests performed with hybrid categories. Moreover, loci L6, L368 and L372, which were outliers at the interspecific level, and in pure *A. anguilla* intraspecific comparisons involving Iceland, were all located in this *A. rostrata* distribution extreme.

Correlations between locus band patterns and explanatory factors in Iceland

Marker states at loci L6, L368 and L372 were also associated with the life stage categorical factor in Iceland (Table 3). For each of these three loci, the frequency of the *A. rostrata* allele showed a significant increase from the glass eel to the yellow eel stage. Moreover, a significant positive correlation was detected with the body length of parental Icelandic yellow eels for loci L6 and L368. In addition, dominant allele frequency at locus L278 was significantly correlated with the latitude of Icelandic sampling locations. Because these correlations could be explained either by an increase in a sample's hybrid proportion with latitude or the transition from glass eel to yellow eel stage (Albert *et al.* 2006), the same tests were also performed after withdrawal of all hybrid categories. Significant relationships were still detected with pure Icelandic *A. anguilla* only (Table 3).

Discussion

The present study aimed to document the possible influence of natural selection on patterns of genetic diversity in Atlantic eels. More specifically, we tested if (i) intra and interspecific genetic variation were influenced by directional selection, and if (ii) interspecific hybrids frequencies could be related to differential survival in Icelandic rivers. We first found candidate loci for directional selection within both species. In *Anguilla rostrata*, the spatial distributions of allelic frequencies displayed by some of these loci were possibly related with the geographical patterns of life-history traits (see below). We also identified a positive correlation between the number of outlier loci and the estimate of neutral differentiation, as well as the latitudinal distance in *A. anguilla*. Second, we found outlier loci at the interspecific level. Non-neutral introgression was also suggested for some loci. Namely, we detected a locus at

Table 3 Results of logistic regressions that revealed significant relationships between allelic composition at a given locus and either categorical (life stage) or continuous variables (body length and sample latitude). Correlations with the life-stage factor were tested using data from the four Icelandic localities for which glass eels and yellow eels were both available. These localities were Vogslækur (VO), Seljar (SE), Vífilsstadavatn (VI) and Stokkseyri (ST) (see Appendix S1). Correlations with body length and sample latitude were tested on all Icelandic localities. Samples consisted of all the individuals available for the localities considered (Pure + hybrids), or were restricted to individuals identified as pure *A. anguilla* (Pure only), or to pure European yellow eels (Pure yellow only). The *P*-value is provided for each test

Locus identifying no.	Factor tested	Localities analysed	Individuals analysed	*P* value
L6	Life stage	VO + SE + VI + ST	Pure + hybrids	1.27×10^{-9}
			Pure only	4.07×10^{-7}
	Body length	All Icelandic	Pure yellow only	7.72×10^{-3}
L368	Life stage	VO + SE + VI + ST	Pure + hybrids	1.23×10^{-7}
			Pure only	1.59×10^{-3}
	Body length	All Icelandic	Pure yellow only	3.27×10^{-5}
L372	Life stage	VO + SE + VI + ST	Pure + hybrids	6.60×10^{-9}
			Pure only	8.56×10^{-8}
	Body length	All Icelandic	Pure yellow only	1.08×10^{-1}
L278	Sample latitude	All Icelandic	Pure + hybrids	6.20×10^{-12}
			Pure only	2.36×10^{-7}

which the typical *A. rostrata* allele hardly crossed the species genetic barrier, whereas three other loci showed accelerated patterns of introgression into *A. anguilla* in Iceland. Moreover, the level of introgression at these three loci increased from the glass eel to the yellow eel stage. We discuss the evolutionary consequences of hybridization in Atlantic eels in the light of these results.

Local differentiation at the species level; evidence for one generation selection footprint?

Regarding the large number of loci studied in a genome scan, one important issue of outlier detection is to deal with type I errors generating false positives. In our case, given the 0.995 threshold used to detect loci under directional selection, we expected 373 * 0.005 or approximately two loci to be detected as outliers only by chance in each comparison. However, the method of Beaumont & Balding (2004) performs a false-positive correction to avoid this issue. Furthermore, our study at the species level involved multiple pairwise comparisons. We could therefore identify outlier loci involved in multiple comparisons, further reducing the likelihood of type I error.

We detected in each species outlier loci that were associated with a particular sample in at least three different pairwise comparisons. Allele frequencies at these markers could have been shaped by locality-specific selective forces. Unfortunately, in the absence of detailed environmental data concerning factors that could play a selective role, no correlation could be examined between outlier locus frequencies and ecological variables. However, geographical patterns in life-history traits can provide a first basis to propose hypothetical explanations for such associations, and set the stage for future research on the role of natural selection in shaping genetic variation observed in eels. Of course, these must be considered as exploratory. For instance, the *A. rostrata* glass eel phase duration is longer and characterized by a slower growth rate in northern localities than in the south (Wang & Tzeng 1998). This latitudinal variation was interpreted as a consequence of coastal water temperature that could influence the speed of glass eels' development. If temperature controls such a vital trait, it could also affect glass eels' mortality, hence providing a basis for differential selection. Atlantic Ocean surface temperatures near North America coasts show that the influence of the warm Gulf Stream water masses does not extend to the north of Boston (see Wirth & Bernatchez 2003 for a map). This raises the hypothesis that AFLP markers, such as L260 that showed unusually high F_{ST} values in all three comparisons involving the northernmost Medomak River, could be candidate for selection by temperature in *A. rostrata*.

In *A. anguilla*, five loci showed unusually high F_{ST} values in at least three different pairwise comparisons when Iceland was compared with European samples (L144, L278, L306, L337 and L372). Icelandic eels are likely to be characterized by some particular traits because they are supposed to have a shorter migration and experience unique ecological conditions during their continental growth compared to their European congeners (Albert *et al*. 2006). Therefore, it was not surprising to find five Iceland-specific outliers among a total of six locality-specific outliers in the European eel. This result supported that the outlier behaviour of such loci was probably related with the existence of selective factors that exclusively operate in the 'outlying locality'.

In both species, some outlier loci were also involved between independent sample pairs, possibly indicating that some selective factors could influence genetic variation on broader geographical scales. This was particularly the case in *A. anguilla*, where a significant positive correlation between latitudinal distance separating European localities and the number of outliers in pairwise comparisons was observed. Due to genetic linkage between genes responsible for local adaptation and some neutral loci, theory also predicts that natural selection could facilitate neutral genetic differentiation via genetic hitchhiking (Charlesworth *et al.* 1997). This was supported by the positive correlation between trimmed F_{ST} values and the number of outliers found in pairwise comparisons between *A. anguilla* localities. This raised the hypothesis that the weak albeit significant neutral genetic differentiation previously reported in the European eel (Daemen *et al.* 2001; Wirth & Bernatchez 2001, 2003; Maes & Volckaert 2002) could be indirectly induced by natural selection associated with a latitudinal environmental gradient along the European coasts. What was previously described as an 'isolation-by-distance' pattern could therefore be interpreted as an 'isolation-by-adaptation' footprint (Nosil *et al.* 2008). However, both processes are not necessarily exclusive. Clearly, a more thorough test of the hypothesis of isolation-by-adaptation as a single generation process will need a wider sampling coverage, and the use of a meaningful set of ecological variables instead of latitude alone in order to identify causal agents of selection.

Genetic architecture of the species barrier

Natural hybridization between Atlantic eels in Iceland provided an opportunity to study the architecture of putative genetic barriers between *A. rostrata* and *A. anguilla*. Depending on the number of genomic regions involved in reproductive isolation, and on their distribution throughout the genome, the permeability of hybrid zones to gene flow can be highly variable (Barton & Hewitt 1985; Kim & Rieseberg 1999; Wu 2001). The AFLP genome-scan approach is a way to concomitantly assess the proportion of loci that flow more or less freely between species and that of 'speciation loci' that hardly cross the species barrier, and are therefore characterized by introgression delays or segregation distortions in hybrids. Most of the following interpretations concerning the different patterns of introgression found at interspecific outlier loci rely on the hypothesis that each species consists of a panmictic or quasi-panmictic pool of individuals. Iceland was also assumed to consist of a mixture of pure European eels and various hybrid crosses, among which F_1, F_2 and BC_{Aan} were probabilistically categorized using NewHybrids. Although the panmictic status of each species is still debated, these assumptions seem reasonable considering the very low levels of intraspecific genetic differentiation evidenced in a previous study (Wirth & Bernatchez 2003).

The low interspecific trimmed F_{ST} value of 0.06 found between pooled continental samples (i.e. without Iceland) was close to the previous neutral differentiation level found with microsatellite markers (0.007–0.040, Wirth & Bernatchez 2003), and indicated either a recent co-ancestry, shared ancestral polymorphism, or a significant level of introgression. Overall, individual admixture proportions determined with Structure (Pritchard *et al.* 2000) in Albert *et al.* (2006), along with the present NewHybrids results, and the 0.135 overall hybrid index found in Iceland, supported the existence of asymmetrical genetic introgression from *A. rostrata* towards *A. anguilla*. Therefore, the European eel's genome seems permeable to the inflow of genes from the American eel. On the other hand, gene flow between species appeared strongly reduced at some AFLP loci after comparison between pooled continental samples. The 8.4% of outlier loci detected between Atlantic eel species was consistently within the 1.5–15.1% range of outliers documented between closely related species in previous studies (Campbell & Bernatchez 2004; Scotti-Saintagne *et al.* 2004; Savolainen *et al.* 2006; Rogers & Bernatchez 2007; Minder & Widmer 2008). Among the 27 interspecific outliers identified, the study of deviation from theoretical frequencies in Icelandic hybrid categories revealed three contrasting locus behaviours. First, in Iceland, five loci showed a strong bias towards parental *A. anguilla* allelic frequencies. Among these, only locus L236 still significantly deviated from theoretical frequencies after Bonferroni correction. This deviation was observed in each hybrid category, and systematically corresponded to the strongest introgression delay of *A. rostrata* allele into *A. anguilla* genome among all markers. Locus L236 is therefore potentially linked with a genomic region undergoing diverging selection between both eel species, or perhaps involved in Dobzhansky–Muller incompatibilities inducing hybrid inferiority (Burke & Arnold 2001). Second, nine interspecific outliers were in conformity with expected allele frequencies in each hybrid category, and only showed moderate departures from neutral expectations using the hybrid index approach. Some of these loci could have been identified by type I errors, as they were not located far above the 0.995 quantile in the single interspecific comparison made for outlier detection. Type I error was however, improbable for three other loci showing a clear interspecific outlying behaviour (L78, L263 and L353). Conformity with theoretical expectations in hybrid categories and in the whole Icelandic sample rather suggested that allelic combinations at these loci did not have a strong influence on hybrid fitness. The interspecific outlier behaviour of such loci may therefore be best explained by divergent selection between parental species unaffecting hybrid fitness. Third, the 13 remaining loci were all distinguished by allelic frequencies biased towards *A. rostrata*, exceeding neutral expectations in at least one hybrid category. Among the seven markers that still significantly deviated after Bonferroni correction, loci L6, L368 and L372 also fell outside the 95% confidence interval defined in the hybrid index approach, suggesting that they were undergoing positive selection in Iceland. Because the overrepresentation of *A. rostrata* alleles at these loci was also independently observed in hybrids and pure Icelandic *A. anguilla*, an Iceland-specific selective factor favouring *A. rostrata*'s alleles in Iceland best explains accelerated introgression at these markers (see below).

On the basis of the behaviour of loci studied at different scales, two main mechanisms may be invoked for shaping the patterns of differential introgression at different markers between Atlantic eel species. Asymmetrical genetic introgression from *A. rostrata* to *A. anguilla* was already demonstrated (Albert *et al.* 2006), but here we found some loci with accelerated rates of introgression in Iceland. Hybridization could thus allow genetic segments carrying neutral but also locally favourable genes to cross the genetic barrier between species. In the face of introgression, genetic swamping would tend to homogenize the genetic composition of the two species, but loci under divergent selection, or potentially involved in endogenous incompatibilities such as L236 could maintain separation of some genomic regions among species. The fact that these regions appeared at low density compared to neutrally flowing loci in this genome scan suggests that the genetic barrier between Atlantic eel species may be very porous (Wu 2001).

Adaptive consequences of hybridization between Atlantic eels

The Iceland-limited occurrence of hybrids was proposed to rely on their intermediate larval behaviour and/or development compared to purebreds (discussed in Albert *et al*. 2006). Because of its geographical localization at mid-distance between North America and Europe and its ecological uniqueness compared to the European core habitat, Iceland can also be considered a marginal habitat of the European eel natural distribution. The restricted distribution of hybrids could indicate that they have higher survival in this habitat compared to purebreds (Albert *et al*. 2006). Contrasted performance between purebreds and hybrids regarding habitat ecology have been proposed in the alpine sedge *Carex curvula*, which maintained its genotype integrity in optimal habitats but showed high levels of introgression at ecological margins (Choler *et al*. 2004). In that study, correlation between patterns of genotypic distribution and ecological conditions suggested that genetic introgression could be a way to widen this species' niche. In such a case, allospecific genes possibly responsible for local adaptation in marginal habitats should introgress more rapidly than any other allospecific neutral gene via introgressive hybridization. Indeed, differential survival is supposed to favour individuals carrying these introgressed-adapted alleles, but should have a random effect on unlinked neutral genes. Loci L6, L368 and L372 are good candidates for such adaptive introgression induced by positive selection, because their allelic compositions were strongly biased towards *A. rostrata* frequencies in Iceland. Moreover, the increase in introgression level observed at these loci during continental growth (i.e. at transition from glass to yellow eel, and during yellow eel growth for L6 and L368) indicated that allelic frequencies changed during the continental phase. The fact that this temporal enrichment in *A. rostrata*'s alleles was not observed at other loci is not compatible with the hypothesis that it reflects a temporal decrease in the introgression rate, because this should also have affected other loci. A segregation distortion induced by co-adapted gene complexes or a spatio-temporal variation in hybrids' proportion could neither be invoked, because the same significant variations in allelic frequencies were also found with pure Icelandic *A. anguilla* individuals. Consequently, we propose that our results reflect the existence of ecological factors driving differential survival in Iceland in favour of introgressed individuals at loci L6, L368 and L372. Moreover, a latitudinal gradient of selection associated with such ecological factors providing an advantage for introgressed individuals at high latitudes could also explain the latitudinal increase in the proportion of hybrids towards the North of Iceland (Albert *et al*. 2006). Although these ecological factors still remain to be identified, temperature would be a logical candidate to investigate in future studies.

It is notoriously difficult to provide evidence for adaptive introgression in natural surveys, because positively selected alleles are predicted to rapidly fix in the introgressed population (Barton 2001). However, the case of Atlantic eels could be particularly useful in this respect, because of the very high levels of neutral gene flow observed at the species scale. The panmixia hypothesis assumes that within each species, alleles are randomly exchanged between individuals at each generation, independent of their continental origin. Therefore, larval pools that recruit at different continental locations should not differ in their allelic composition when starting to disperse from the spawning area. As larval transport by the Gulf Stream is likely to be an additional source of genetic homogenization during dispersal, only oceanic selection could differentially shape genetic variation among pools of recruiting larvae. The eventual footprint of positive selection observed in Iceland could therefore correspond to a single generation process.

Conclusions and perspectives for future research

This genome scan provided evidence that both intraspecific and interspecific genetic diversity are influenced by natural selection in Atlantic eels. Genetic differentiation patterns found at the species scale suggested that differential mortality associated with local conditions where young eel settle could indirectly shape neutral genetic variation via genetic linkage with loci under selection. Hence, the nature of genetic variation in Atlantic eels could be not only spatial or temporal, but also ecological. In order to provide a better understanding of some novel aspects raised in this study, a genome-wide functional single nucleotide polymorphism (SNP) scan could enable the identification of coding genes under potential selection following preliminary efforts to increase SNP discovery (Namroud *et al*. 2008). Wood *et al*. (2008) also recently showed that potentially selected regions can fall outside coding sequences, and that BAC library screening was an appropriate way to follow up an AFLP genome scan. Such markers and approaches could be applied towards further investigating the effect of natural selection on changes of allele frequencies at putative 'adaptive genes' throughout the eel's complex life cycle. Namely, the comparison between leptocephali of different ages could help in understanding the impact of selection on genetic variation in the marine environment, whereas the analysis of glass and yellow eels would provide information on selection operating in continental waters, and as such, enable estimation of the nature and relative importance of selection operating either in the marine or continental environments.

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P.A. Gagnaire's current research involves the investigation of population structuring and speciation processes in the genus *Anguilla*. The work presented in this paper was initiated while visiting L.B.'s laboratory. V. Albert is a research assistant in L.B.'s laboratory specialized in population genetics. B. Jonsson's interests are in the field of evolutionary ecology, effects of climatic change and life history adaptation. Louis Bernatchez's research focus on understanding processes of molecular and organismal evolution and their significance for conservation.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Map showing the geographic distribution of the sampling sites in this study.