Replicated anthropogenic hybridisations reveal parallel patterns of admixture in marine mussels.

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Anthropogenic activity can create new contacts between genetically differentiated lineages, which start to exchange genes again before our eyes. When similar introductions occur in different places, they provide replicated large-scale hybridisation experiments. We studied the population genetics of *Mytilus* mussels in several *M. galloprovincialis* introduction areas. Using a dataset of 4279 mussels, genotyped at 77 ancestry informative markers, we document the introduction of the Mediterranean lineage of *M. galloprovincialis* to five ports in France and of the Atlantic lineage in Norway. In each case, the introduced species has extensively admixed with *M. edulis*. In Ports, we also observed sharp genetic shifts, at a very fine spatial scale, at the open sea-port interface, where the seaward native population is *M. edulis*. The sole exception was the bay of Brest, where the native population is the Atlantic lineage of *M. galloprovincialis*, and where the invading mussels started to spread and admix with the native background. Sharp transitions at the port entrance, at scales below the dispersal distance, do not fit well with the predictions of a migration-selection tension zone model, and instead suggest habitat choice and adaptation to the environment of the port, possibly coupled with connectivity barriers. We then examined departures from the average admixture rate at different loci (i.e., Barton's concordance). We compared the two human-mediated admixture events (French ports and Norway), to naturally admixed populations, and to transmission ratio distortions observed in experimental lab crosses. When the same *M. galloprovincialis* background was involved, we found positive correlations in the departures of marker loci in the different locations; but when different *M. galloprovincialis* backgrounds were involved, we found no or negative correlations. Some of the positive correlations we observed are best explained by a shared history during saltatory colonisation, but some independent replicates imply parallel selective processes, suggesting that the genome-wide effect of admixture is highly repeatable.

Keywords: biological introductions, bentho-pelagic species, ports, secondary contact, clines, admixture, genomics.

1 Introduction

Biological introductions have evolutionary impacts on both native and introduced species, and through both ecological and genetic responses (Mooney and Cleland 2001; Strayer et al. 2006; Prentis et al. 2008; Suarez and Tsutsui 2008). Importantly, as a component of "anthropogenic hybridizations" (see McFarlane and Pemberton 2019 for a recent review), human-mediated introductions can alter the genetic composition of native and introduced species. Such processes are likely to be common, and probably underestimated (Largiadèr 2008). Anthropogenic hybridisation has been reported in diverse taxonomic groups, including plants, birds, fishes, mammals and invertebrates (Largiadèr 2008 and references therein). For instance, in nineteen different fish families, half of the observed interspecific hybridisations have been attributed to human disturbances (Scribner et al. 2000). The outcomes of these hybridisations could be similarly diverse. Hybridisation might favour the sustainable establishment of non-indigenous species (NIS) by facilitating adaptation to the local environment via the introgression of "ready-to-use" alleles from native genomes. Immediate advantage could also be gained through heterosis at the initial stage of introduction (Ellstrand and Schierenbeck 2000; Suarez and Tsutsui 2008; Schierenbeck and Ellstrand 2009). Conversely, from the native species' standpoint, hybridisation is often considered as "genetic pollution", raising concerns of "extinction by hybridization and introgression" (Rhymer and Simberloff 1996); although these concerns often underrate the positive effects of genetic rescue on the native populations (Harris et al. 2019). Additionally, hybrid fitness depression might oppose introduction success (Kovach et al. 2016) and favour the confinement of the introduced lineage, perhaps by trapping the introduced-native boundary at a natural barrier (Barton 1979). Overall, the evolutionary consequences of anthropogenic hybridisation (i.e., gene flow, local introgression, reinforcement, or rescue) are likely to be strongly contingent on intrinsic and extrinsic factors, such as the accumulation of reproductive incompatibilities or local selection processes (Abbott et al. 2013).

While evolutionary processes undoubtedly play a major role in the biological invasion process, introductions with hybridisation can also shed light on the evolutionary process itself. Natural hybrid zones have been extensively used for the study of speciation (Barton and Hewitt 1989; Abbott et al. 2013). In Hewitt's 1988 words, human-induced hybrid zones, like natural hybrid zones, can be seen as "natural laboratories for evolutionary studies". Anthropogenic introductions have a special value because they tend to be recent, and replicated, and can often be compared to natural admixture events between the same lineages (Bouchemousse et al. 2016). This is important because, even with genome-wide genetic data and powerful inferential methods, the traces of secondary contacts tend to erode over time, and can be confounded with other processes (Bierne et al. 2013; Bertl et al. 2018). Recent secondary contacts allow a unique window on the processes involved, including the sorting of alleles in admixed populations (Schumer et al. 2018).

The blue mussel complex of species (*Mytilus* spp.) is composed of three species which are incompletely reproductively isolated despite several million years of divergence, and readily hybridise where they meet (Roux et al. 2014). *M. edulis* (Linnaeus 1758), *M. galloprovincialis* (Lamarck 1819) and *M. trossulus* (Gould 1850) are naturally distributed in temperate regions of the northern hemisphere. Where found in sympatry, the distribution of *M. edulis* and *M. galloprovincialis* are correlated with salinity, tidal height and wave exposure (Gardner 1994; Bierne et al. 2002b). In certain cases, *M. edulis* occupies sheltered, deeper or estuarine environments, while *M. galloprovincialis* is found on more wave-exposed parts of the coast. In regions with a single species, however, individuals can occupy all niches. It should also

be noted that independent contacts can show reversed associations with the environment, in agreement with the coupling hypothesis (Bierne et al. 2011). The species *M. galloprovincialis* referred to as the Mediterranean (Med.) mussel has a large natural distribution – from the Black Sea to the North of the British Isles – and has been shown to be divided into two main lineages, Atlantic (Atl.) and Med. (Quesada et al. 1995b; Roux et al. 2014; Fraïsse et al. 2016). Those two lineages form hybrid zones in the Almeria-Oran front region (Quesada et al. 1995a; Quesada et al. 1995b; El Ayari et al. 2019).

Mussels have several traits making them prone to transportation by humans. This bentho-pelagic mollusc has a notably long-lived larval stage offering possibilities for long distance spread through both marine currents (Branch and Steffani 2004) and anthropogenic means, including in ballast water (Geller et al. 1994) or as a fouling organism (e.g. on hulls: Apte et al. 2000; Casoli et al. 2016, or on marine litter: Miller et al. 2017; Miralles et al. 2018; Węsławski and Kotwicki 2018). Mussels are also heavily cultivated on a global scale (287,958 tonnes in 2016, FAO 2018). They can therefore be introduced by the two main introduction pathways of marine species: international shipping and aquaculture (Molnar et al. 2008). While larval dispersal might allow a post-introduction range expansion, the initial establishment is rendered difficult by a strong demographic and genetic Allee effect. Therefore, successful establishment is dependent either on a large propagule pressure, likely to occur in many marine NIS (Rius et al. 2015; Viard et al. 2016), or on hybridisation with a native species, which would counterbalance Allee effects (Mesgaran et al. 2016). *Mytilus* mussels display both high fecundity and high density traits, associated with incomplete reproductive isolation, allowing them to escape such effects. Due to its hybridisation and invasive potential, the *Mytilus* species complex is a model of choice when investigating the genetic evolutionary consequences of marine invasions (Saarman and Pogson 2015, Popovic et al. this issue).

Among *Mytilus* species, *M. galloprovincialis* has been introduced many times across the globe, in both the northern and southern hemispheres, and notably, along the Pacific coast of North America, in South America, South-Africa, Asia, and Oceania. (McDonald et al. 1991; Daguin and Borsa 2000; Branch and Steffani 2004; Kartavtsev et al. 2005; Saarman and Pogson 2015; Han et al. 2017; Larraín et al. 2017). By contrast, we only know of a few cases of *M. edulis* ingressions – either aborted or successful – into non-native areas (Crego-Prieto et al. 2015; Casoli et al. 2016; Fraïsse et al. 2018a), and one case of successful *M. trossulus* introduction (Beaumont et al. 2008). Branch and Steffani (2004) reported that observed introductions of *M. galloprovincialis* happened close to large shipping ports with a secondary range expansion from these points. For instance in South-Africa, *M. galloprovincialis* spread rapidly and had negative and positive impacts on local communities, modulated by wave action (Branch and Steffani 2004; Branch et al. 2008). Wherever *Mytilus* species are native, *M. galloprovincialis* has been shown to be highly competitive and has often displaced local mussels (Carlton et al. 1999). *M. galloprovincialis* has also been reported in the subarctic and Arctic, notably in Norway (Brooks and Farmen 2013; Mathiesen et al. 2016). Given the low divergence between Atl. and Med. *M. galloprovincialis*, and their assignment to the same species, introduced *M. galloprovincialis* has often been reported without further investigation of its origin, and when markers are insufficiently informative, the origin is necessarily unresolved. Nevertheless, it is clear that both lineages have been successfully introduced in multiple places worldwide (Atl.: South-Africa, Australia; Med.: Eastern and Western Pacific, Australia – Daguin and Borsa 2000; Han et al. 2017; Zardi et al. 2018; Popovic et al. this issue).

Ports are considered to be particularly prone to the introduction of alien species and their maintenance (Bax et al. 2002). Because they are hubs of maritime traffic, with high connectivity, they are bridgeheads towards expansion at regional scale (Drake and Lodge 2004). Vessels

have been shown to be a major introduction pathway, through various vectors, including ballast water, sea-chest and hull (Sylvester et al. 2011; Katsanevakis et al. 2013). In addition, ports are often distinct from nearby natural habitats, with particular environmental features. These new niches can be colonised by opportunistic species, such as many NIS (Bishop et al. 2017, and references therein). With their life history traits, and high tolerance for environmental fluctuations, mussels are likely to be introduced and become established in ports.

In this study, using a population genomics dataset comprising 4279 mussels genotyped at 77 ancestry informative SNPs, we examined mussel populations established in ports in North-West France (located along the Atlantic and the English Channel coastlines), and compared these to mussel populations established in the vicinity. This genetic survey allows to report, for the first time, an unexpected and extensive introduction of the Med. lineage of *M. galloprovincialis* into five ports in our study area. In addition, we show that the introduced mussels – henceforth "dock mussels" – are genetically unique and originate from an admixture event with native *M. edulis*. Dock mussels additionally form small-scale hybrid zones with native mussels at the sea-port interface, which can be either *M. edulis* or Atl. *M. galloprovincialis* depending on the port. Port populations appear to constitute stable admixed populations. We additionally reanalysed previously published (Mathiesen et al. 2016) and new samples of putative *M. galloprovincialis* in Norway, and concluded that they are introduced Atl. *M. galloprovincialis* admixed with the local Northern European (North-Eu.) *M. edulis* lineage. We combined our data with multiple samples of admixed populations from natural hybrid zones and lab crosses. This allowed us to compare multiple independent events of admixture.

The similarities and differences between these scenarios help to clarify the factors that determine the outcome of an introduction with hybridisation. In particular, we show that similar outcomes sometimes reflect shared colonisation history, but can also arise in truly independent colonisations. However, this predictability is highly background dependent, and replicated outcomes only appear when the same parental backgrounds are involved.

2 Methods

2.1 Sampling and genotyping

We either received samples from collaborators or directly sampled in the areas of interest (see fig. S1 for a map presenting all samples and tab. S1). Most of the port sites were sampled between 2015 and 2017 and older samples were included as references or for temporal information.

We re-genotyped samples from published studies where the presence of *M. galloprovincialis* was detected without identification of the Atl. or Med. *M. galloprovincialis* origin. These published studies are: (i) Mathiesen et al. (2016) who studied the genetics of *Mytilus* spp. in the sub-Arctic and Arctic using 81 randomly ascertained SNPs. They identified *M. galloprovincialis* and putative hybrids with *M. edulis* in the Lofoten islands, Svalbard and Greenland. Their parental reference samples included only the Atl. lineage of *M. galloprovincialis* (Galicia, Spain). Our aim was to further assess the origin of the *M. galloprovincialis* ancestry. (ii) Coolen (2017) studied samples from the North Sea with 6 microsatellite markers and the locus Me15/16. The objective was to study connectivity between offshore energy installations. They identified populations containing individuals with *M. galloprovincialis* ancestry, using an Atl. *M. galloprovincialis* reference as well (Lisbon). We reanalysed 9 sites from their study. Additionally another oil platform sample from the Norwegian sea (Murchison oil station, MCH) and one Norwegian sample (Gåseid, GAS) were included.

Crosses produced in Bierne et al. (2006) between *M. edulis* and Med. *M. galloprovincialis* were included in this study to serve as an independent replicate of hybridisation comparable to admixtures found in ports. Briefly, F1 hybrids were first produced by crossing five males and five females of *M. edulis* from Northern France and *M. galloprovincialis* from the western Mediterranean sea (Thau lagoon, France). F2s were produced by crossing one F1 female and five F1 males. Additionally, sex-reciprocal backcrosses to *M. galloprovincialis* were made, they are named BCG when the females were *M. galloprovincialis* and BCF1 when the female was F1. Crosses production are thoroughly described in Bierne et al. (2002a), Bierne et al. (2006), and Simon et al. (2018).

We sampled gill, mantle or hemolymph tissues from mussels either fixed in 96% ethanol or freshly collected. We used the NucleoMag™ 96 Tissue kit (Macherey-Nagel) in combination with a Kingfisher Flex™ (serial number 711-920, ThermoFisher Scientific) extraction robot to extract DNA. We followed the kit protocol with modified volumes for some reagents: beads 2× diluted in water, 200 L of MB3 and MB4, 300 L of MB5 and 100 L of MB6. The extraction program is presented in fig. S2.

Genotyping was subcontracted to LGC genomics (Hoddesdon, UK) and performed with the KASP™ method (Semagn et al. 2014). We used a set of ancestry informative SNPs developed in (Simon et al. 2018, Simon et al. in prep.). For cost reduction, we used a subset of those SNPs while ensuring they were still sufficient for species and populations delineations. Multiple experiments of genotyping were performed. The results were pooled to obtain a dataset made of 81 common markers.

2.2 Filtering

Analyses were carried out using R (v3.5.1, R Core Team 2018) and custom Python 3 scripts for format conversions. Software versions and used packages are listed in table S2. Decision thresholds for all analyses and dataset selections are summarised in table S3.

First, control individuals duplicated between genotyping experiments were removed by keeping the one having the least missing data. Over 81 markers, the maximum number of mismatches observed between two duplicated individuals was 2 (without considering missing data), showing that the genotyping method is mostly accurate. A few individuals identified as affected by a *M. trossulus* transmissible cancer were removed from the dataset (Metzger et al. 2016; Riquet et al. 2017).

The dataset was filtered for missing data with a maximum threshold of 10% for markers over all individuals and 30% for individuals over all markers. This filtering yielded 4279 individuals genotyped at 77 loci (from 4495 individuals for 81 loci). We separated nuclear (76 loci) and mitochondrial (1 locus) markers for downstream analyses. The mitochondrial marker (named 601) is located on the female mitochondria.

Most analyses required reference population samples. A list of reference individuals and groups (fig. 1C and tab. S4) was set *a priori* using the literature and our knowledge of the *M. edulis* species complex. We defined three levels of structure that we call L1, L2 and L3. L1 is the species level comprising *M. edulis* (edu), *M. galloprovincialis* (gallo) and *M. trossulus* (tros). L2 defines allopatric lineages in each species: (i) American (edu_am, East coast) and European (edu_eu) *M. edulis*; (ii) Atl. (gallo_atl) and Med. (gallo_med) *M. galloprovincialis*; (iii) Pacific (tros_pac), American (tros_am East coast) and European (tros_eu, Baltic sea) *M. trossulus*. Finally, L3 defines sub-populations where the differentiation is mainly due to local introgression following historic contacts between lineages (Fraïsse et al. 2016): (i) North-Eu. populations of *M. edulis* (edu_eu_north) were included (Simon et al. in prep.), this

lineage is present along the coast of Norway and meet with edu_eu_south along the Danish coast; (ii) Atl. *M. galloprovincialis* from the Iberian peninsula (gallo_atl_iber) and mussels from Brittany (gallo_atl_brit); (iii) West (gallo_med_west) and East (gallo_med_east) Med. *M. galloprovincialis*, the limit being set at the Siculo-Tunisian strait.

To improve this predefined set of reference samples, an initial genetic clustering was performed with the software `Admixture` (Alexander et al. 2009, full nuclear dataset, $K = 3$, 30 replicates, fig S4) and the results were combined with the `CLUMPAK` software (Kopelman et al. 2015). All individuals with less than 85% ancestry from their putative cluster were removed from the reference set. (This threshold was chosen to account for local introgression in some populations.) This step ensures there are no migrants, either from introduction or from sympatric species, and no hybrids in the reference panel.

Once the reference dataset was established, Hardy-Weinberg equilibrium (HWE) was estimated in each L3 level for all markers. edu_eu_south was separated in two groups, corresponding to the bay of Biscay (int, as in Fraïsse et al. 2016) and the English Channel (ext) for this analysis only as they do not mate randomly but do not show significant genetic differentiation (tab. S6). We used the `hw.test` function of the R package `pegas` with 10^4 Monte Carlo permutations and a Benjamini-Yekutieli false discovery rate correction. Markers 604 and 190 were identified as significantly departing from HWE in at least one reference group (fig. S3).

2.3 Genetic map

Estimates of linkage between markers allows us to account for admixture linkage disequilibrium in ancestry estimation (see `Structure` analyses below), and to estimate time since admixture.

We used F2 crosses to produce a genetic map for a subset of markers analysed by Simon et al. (2018). This dataset comprises 97 markers genotyped for 110 reference *M. edulis* individuals, 24 reference Med. *M. galloprovincialis* individuals, 6 F1 parents (1 female, 5 males) and 132 F2 offspring. Markers that were not heterozygote in all F1 parents, or with an allele frequency difference between species lower than 0.2 were filtered out to avoid spurious distortions and correct orientation. We also removed two markers with >10% missing data. This left a final dataset of 40 informative markers, and 114 F2 offspring. Alleles were oriented according to their frequencies in reference samples. We then used the R package `qtl` to produce a genetic map. Four additional markers were dropped by the internal checks in the package, for not passing the Mendelian segregation test in F2s (with Holm-Bonferroni correction). The final genetic map comprises 36 markers scattered among 16 linkage groups (tab. S5). Only the first 8 linkage groups contain more than one marker.

An "unlinked" set of markers was created by keeping the marker with the least missing data in each linkage group or physical contig. Markers not included in the linkage map analysis were considered to be unlinked. See table S5 for a list of unlinked markers.

2.4 Population differentiation and genetic clustering

Population differentiation was used to assess the power of our set of ancestry-informative markers, and to test differences between introduced populations. Genetic clustering was then used to classify individuals to known lineages or assess levels of admixture in the studied populations.

Hierarchical population differentiation tests were carried out with the R package `hierfstat` (Goudet 2005). We used 10^4 permutations for all tests. The Weir and Cockerham F_{ST} estimator is reported when presenting population differentiation results. When calculating

population differentiation between reference groups, markers with more than 30% missing data in *M. trossulus* populations were removed because of badly typed markers in this species (tab. S3).

Ancestry estimation was performed with the Bayesian model implemented in the program `Structure` (Falush et al. 2003), which includes additional models of interest compared to the aforementioned `Admixture` software. Each result is composed of 25 replicates for each K, run for $8 \cdot 10^4$ steps after a $2 \cdot 10^4$-step burn-in. All analyses use uncorrelated allele frequencies (FREQSCORR = 0) and a separate and inferred α for each population (POPALPHAS = 1, INFERALPHA = 1, Wang 2017). Replicates were merged with the program `CLUMPAK` (default parameters with MCL threshold set at 0.7).

For `Structure` analyses, markers that departed from Hardy-Weinberg equilibrium in focal reference populations were removed to avoid departure from the algorithm model. The program was either run using the admixture model with linkage with the F2 genetic map generated, or using the no-admixture model with the unlinked dataset (tab. S5), as both models cannot be used simultaneously.

A first `Structure` analysis on the full dataset was used to remove all individuals with *M. trossulus* ancestry to focus on a "reduced dataset" of *M. edulis* and *M. galloprovincialis*. Because, *M. trossulus* is present in sympatry in Norway and can hybridise with its congeners, a threshold of 10% ancestry was used to identify parental and most recent hybrid individuals. From this reduced dataset, two analyses – with and without the admixture model – were performed ($K = 4$). Additionally, to allow a better classification of individuals at bay scales, `Structure` analyses were performed on a "local dataset" with the ports and surrounding populations, with and without admixture, and without including the reference populations. Finally, `Structure` runs targeted at estimating the age of the admixture were used (see supplementary methods for details). The number of generations was estimated as presented in Falush et al. (2003).

Mussels from the admixed populations with Atl. *M. galloprovincialis* (introduced and natural) were classified using the reduced dataset without admixture ($K = 5$, fig. S19). To obtain a finer classification in port areas, mussels were assigned to *M. edulis*, Atl. *M. galloprovincialis* or dock mussels clusters using the local `Structure` analysis without admixture ($K = 3$, fig. S20). See table S3 for details on the selection thresholds for each group and figure S21 for independent plots of selected individuals.

The software `Newhybrids` (Anderson and Thompson 2002) was used to evaluate the probability of individuals to belong to classes of first generation hybrids between the dock mussels and native lineages (fig.S25-S26).

2.5 Levels of ancestry comparisons

For each population of interest, admixed individuals (identified as described above) were kept and native and/or parental individuals were removed. The $K = 4$ `Structure` output was used for those comparisons (fig. S21), allowing to separate the four clusters: edu_eu_south, gallo_atl, gallo_med and edu_am. This selection allows an homogeneous comparison of ancestry levels between all admixed populations (fig. S22).

For each `Structure` cluster, a Kruskal-Wallis one-way ANOVA was used to compare multiple populations. Additionally, post-hoc pairwise comparisons were carried out using a Dwass-Steel-Crichtlow-Fligner test with Benjamini-Yekutieli corrections for multiple testing.

Independent comparisons were used between ports and between admixed populations with Atl. *M. galloprovincialis*. Additionally Atl. *M. galloprovincialis* from Brittany were compared

between three groups: (i) distant from the bay of Brest individuals, (ii) individuals outside the bay of Brest (the limit being the entrance straight), and (iii) individuals inside the bay of Brest.

2.6 Least cost distance analyses and Geographic cline fitting

As a proxy for connectivity between sampling sites, least cost path distance matrices were produced for each port allowing to take into account obstacles such as land and human made barriers (e.g., breakwaters and seawalls). A raster of costs was built for each port from polygon shapefiles ("Trait de côte Histolitt Métropole et Corse V2", produced by SHOM and IGN) modified to include small port structures that could stop larval dispersal or to exclude inaccessible parts. Locks inside ports were considered as opened for the purposes of distance calculation between isolated points. We used the program QGIS to handle polygons and raster creation. Land was coded as missing data and water was set with a conductance of one. The R package `gdistance` was used to compute transition matrices based on those cost rasters and to compute least cost distances between points for each dataset.

Transitions at the sea-port interface can be investigated at the locus level by fitting clines of allele frequencies along a spatial axis. The objective is to assess the concordance of transitions between markers and with the observed global ancestry.

Geographic clines were fitted for each port (except Saint-Malo which only has one port sample) with the R package `hzar`. The port of Le Havre was divided into two transects: North and South corresponding to the historic basins and the "Port 2000" recent installations respectively. The least cost distance from the most inward site in each port (indicated by a triangle in fig. 3) was taken as a proxy for geographic distance and to project geographic relationships on a single axis. For the bay of Brest, the starting site was taken as the right-most population in figure 1G, up the Élorn estuary. The three points in the bottom-right corner of figure 3E containing Med. *M. galloprovincialis* ancestry were excluded from the fit, to account for discrepancies between least cost path distances and the presence of the introduced background. Clines were fitted using a free scaling for minimum and maximum frequency values and independence of the two tails parameters. We used a burn-in of 10^4 and a chain length of 10^5 for the MCMC parameter fit.

2.7 Distortions from expected frequencies and correlations

Our data include multiple admixture events. To ask if outcomes were similar across events, we compared the deviations of marker allele frequencies from their expected values in each situation.

We denote the expected frequency of an allele in an admixed focal population as f_{exp}. This expected value is calculated from the observed allele frequencies in pure-lineage reference populations, and from the mean ancestry values across all markers for the focal population, as estimated from `Structure`.

Admixed population frequencies are calculated only with admixed individuals in each population (see section 2.5 for details and fig. S21 for selected individuals). We used the results of ancestry estimation from `Structure` with $K = 4$ clusters (edu_eu_south, gallo_atl, gallo_med, and edu_am) and summed ancestries from South-Eu. and American *M. edulis*, giving the composite ancestry estimation Q_{edu} for each individual:

$$Q_{edu} = Q_{edu_eu_south} + Q_{edu_am} \tag{1}$$

In particular, with three reference populations, the expected allele frequency is:

$$f_{exp} = f_{local\ edu} \cdot \overline{Q}_{edu} + f_{gallo_atl} \cdot \overline{Q}_{gallo_atl} + f_{gallo_med} \cdot \overline{Q}_{gallo_med} \quad (2)$$

Here, the f values denote the allele frequencies in the reference population indicated by the subscript, and the Q values denote the mean ancestry from the focal admixed population. gallo_med and gallo_atl correspond to the L2 level encompassing lower population classifications (fig 1C and tab. S4) as the origin of the introduced population is not known below this level.

For lab crosses, the parental Med. *M. galloprovincialis* L3 level is known and correspond to gallo_med_west. Therefore its frequency was used in place of $f_{\text{gallo_med}}$. For dock mussels the "local edu" lineage is taken to be edu_eu_south. For LOF and GAS admixed populations, we used the North-Eu. *M. edulis* lineage (edu_eu_north) to estimate parental allele frequencies ($f_{\text{local edu}}$) while using the usual $\overline{Q}_{\text{edu}}$ estimation.

The deviation of the observe frequency f_{obs} from the expected frequency f_{exp} is denoted D:

$$D = f_{obs} - f_{exp} \quad (3)$$

This computation allows us to estimate a distortion by locus from the average genomic expectation given the population ancestry and parental allele frequencies. The correlation of distortions by locus are then correlated between admixed populations, corresponding to different admixture events (e.g. between one port and one introduced Atl. *M. galloprovincialis* population). For each correlation, we used Pearson's r to estimate the strength of the correlation and tested the significance with a permutation test ($5 \cdot 10^4$ permutations). The classic t-test was not used due to the distortions not following normality.

When multiple correlations pertained to the same null hypothesis (e.g. that distortions in lab backcrosses do not correlate with distortion in ports), and datasets contained possible non-independence (e.g., from migration of hybrids between ports), we used a modified Fisher's method to combine p values, developed by Poole et al. (2016) and implemented in the R package `EmpiricalBrownsMethod`.

3 Results

The sampling for this study focused on populations where we had *a priori* expectations of admixture. Therefore, it should not be confused with a representative sample of the *M. edulis* complex, where populations are usually much closer to the reference parental populations. With this in mind, we began with the discovery of mussels with Med. *M. galloprovincialis* ancestry in the port of Cherbourg, as sampled in 2003 (Simon et al. in prep.). We then sampled comparable ports and neighbouring natural populations. Additionally, natural samples were included from locations where *M. galloprovincialis* alleles had been previously found (e.g. Mathiesen et al. 2016; Coolen 2017), including samples genotyped for other purposes. Throughout the manuscript, we refer to "dock mussels" which correspond to introduced Med. *M. galloprovincialis* in the five ports (Le Havre: havre, Cherbourg: cher, Saint-Malo: stmalo, Brest: brest, Saint-Nazaire: stnaz), to introduced Atl. *M. galloprovincialis* from Norway (Lofoten: LOF and Gåseid: GAS) and to naturally admixed (as far as we know) Atl. *M. galloprovincialis* from four different populations (Aberdeen, Scotland: ABD; Murchison oil station: MCH; Jersey, France: JER; hybrid zone from the South of Brittany: HZSB). The South of Brittany hybrid zone (HZSB) is composed of the following populations: Mousterlin point (MOU), La Jument (JUM), Barres de Pen Bron (PEN), Chemoulin point (CHE), Groix, Penestin (BIL_001), Le Pouliguen (POU_001), and Houat Island (HOU_001).

3.1 Population structure

3.1.1 Differentiation between reference and admixed populations

An initial `Structure` analysis with all samples showed that *M. trossulus* is present in only a few samples (i.e. Norway, North Sea), where its presence was predictable (fig. S5). Because they are not centrally relevant to the present work, individuals with more than 10% ancestry from the *M. trossulus* genetic background were removed.

Hierarchical genetic differentiation tests based on putative groups (see fig. 1C and tab. S4 for groups) showed significant F_{ST} distances until level L3. F_{ST} ranges between 0.72 and 0.81 between species (L1), between 0.38 and 0.48 for L2 levels within species and between 0.0024 and 0.31 for L3 levels inside L2 (see tab. S6 for details). Note that our SNP panel is enriched for ancestry-informative SNPs and these values should not be interpreted as genome-wide averages.

Figure 1 presents the three first components of a Principal Component Analysis (PCA) carried out on a subset of populations including references, port samples (close to or inside ports), introduced Atl. *M. galloprovincialis* in Norway (LOF and GAS), naturally admixed Atl. *M. galloprovincialis* (ABD, MCH, JER and HZSB) and lab crosses samples. The SNP panel used discriminates *M. edulis* from *M. galloprovincialis* on the first axis and Atl. from Med. *M. galloprovincialis* on the second axis (fig. 1A). The third axis discriminates American from European *M. edulis*, with the North-Eu. lineage as an intermediate cluster (fig. 1B). Figure 1C presents a schematic dendrogram of the relatedness between *Mytilus* species and lineages, as well as known introgressions.

Port samples belong to four categories in figures 1A-B: (i) Pure Southern European (South-Eu.) *M. edulis* corresponding to the native mussels found in Cherbourg, Le Havre and Saint-Nazaire. (ii) Pure Atl. *M. galloprovincialis* from Brittany corresponding to the native mussels in Brest, Saint-Malo and Saint-Nazaire. (iii) Intermediate individuals between Atl. *M. galloprovincialis* and *M. edulis* corresponding to admixed individuals and hybrids in either the bay of Brest area, Saint-Nazaire or Saint-Malo which are all in or close to natural hybrid zones between those two species. Additionally, the aquaculture of *M. edulis* in the bay of Brest, imported from the Bay of Biscay, is a substantial source of *M. edulis* in this area as escapees from cultivated sites (and their natural progeny) are common (for details see fig. S11). (iv) A large cluster distributed between Med. *M. galloprovincialis* and *M. edulis*. This cluster, intermediate between the two aforementioned backgrounds, is exclusively composed of mussels sampled in ports. It is not differentiated on another axis of the PCA. This cluster corresponds to "dock mussels" in what follows. These dock mussels appear to be closer to Med. *M. galloprovincialis* than they are from *M. edulis*. This cluster shows a large variance in all directions, presumably including hybrids with both *M. edulis* and Atl. *M. galloprovincialis*. It is noteworthy that apart from lab crosses (F1, F2 and BCs) between Med. *M. galloprovincialis* and South-Eu. *M. edulis*, no other samples cluster in this region of the PCA. Indeed, the mixing of those genetic backgrounds would not be expected without human intervention, as their ranges do not overlap (Fig. 2). The range of the Med. *M. galloprovincialis* lineage being naturally restricted to the Mediterranean sea, we conclude that dock mussels have been introduced in the sampled ports.

Finally, samples from Scotland (ABD), the English Channel Island of Jersey (JER), the Murchison oil platform (MCH) and the natural hybrid zone in South Brittany (natural_atl category HZSB, fig. 1) are intermediate between Atl. *M. galloprovincialis* and South-Eu. *M. edulis*. Those four populations possess overall a large variance and covers much of the range between the two mentioned parental species. Jersey (JER) is the most *M. edulis* population

while Murchison (MCH) is the most *M. galloprovincialis* one (see fig. S22 for details). Norway samples on the other hand are intermediate between Atl. *M. galloprovincialis* and North-Eu. *M. edulis*, suggesting an independent admixture event with the local native lineage.

3.1.2 Bayesian estimation of ancestry

We used the Bayesian clustering algorithm to classify individuals into known genetic backgrounds or obtain their ancestry compositions. The number of clusters (K) was chosen on the basis of the most parsimonious result according to *prior* knowledge of the population structure obtained with our ancestry informative panel of markers.

Our main `Structure` results are presented spatially in figure 2 and 3 (See fig. S6-S15 for the complete dataset). Reference samples are plotted in figure 2 along the four admixed populations studied: Jersey (JER, France), Aberdeen (ABD, Scotland), Gåseid (GAS, Norway) and Lofoten (LOF, Norway). Figures 3A-F detail the geographic distribution of dock mussels in the five French ports. The studied ports are localised in regions characterised by different native species (fig. 2). The native species around Le Havre and Cherbourg is *M. edulis* while in the bay of Brest, the native mussels are Atl. *M. galloprovincialis*. Saint-Malo and Saint-Nazaire lie on the limits of hybrid zones between *M. edulis* and *M. galloprovincialis*. However, surroundings of Saint-Malo are mostly inhabited by Atl. *M. galloprovincialis*, and Saint-Nazaire is located in a zone mostly composed of *M. edulis* with the presence of Atl. *M. galloprovincialis* in sympatry. Here, local *M. galloprovincialis* are more introgressed by *M. edulis* than those found in Brittany as they lie at the far end of the South Brittany hybrid zone (Bierne et al. 2003).

On average, dock mussels are composed of 25% Eu. *M. edulis* ($SD = 11.17$, $N = 879$), 69% Med. *M. galloprovincialis* ($SD = 11.85$), 4% Atl. *M. galloprovincialis* ($SD = 6.08$) and 2% American *M. edulis* ($SD = 3.04$). Introduced Atl. *M. galloprovincialis* are composed of 40% Eu. *M. edulis* ($SD = 15.82$, $N = 63$), 16% American *M. edulis* ($SD = 15.35$), 41% Atl. *M. galloprovincialis* ($SD = 13.91$), and 3% Med. *M. galloprovincialis* ($SD = 3.83$).

Importantly, dock mussels are all localised inside port infrastructures, and we observed a sharp shift at the sea-port interface. For the ports of Saint-Nazaire, Saint-Malo, Cherbourg and Le Havre only four individuals with Med. ancestry were detected in wild populations (out of 341 individuals presented in fig. 3). Those individuals were observed at distances between a few hundred meters to 30 km. Four of the five studied ports (all except Brest) have locked basins where introduced mussels were found.

The bay of Brest (fig. 3E-F) is of particular interest for two reasons. First, the local background is the Atl. lineage of *M. galloprovincialis*, contrasting with the other ports where the native background is *M. edulis*. Second, the introduced mussels have been detected outside port infrastructures, which motivated an extensive sampling. Dock mussels are restricted to the inside of the bay with no detectable influence on external *M. galloprovincialis* populations. Contrary to the other ports, introduced mussels extensively colonised the local environment, mainly close to estuarine areas.

For the port of Cherbourg, we were able to analyse several temporal samples between 2003 and 2017 (fig. 3B). These exhibit a small differentiation between the 2003 sample and later years (2015 and 2016; $F_{ST} = 0.0066$ and 0.0097, tab. S8) and this seems to be driven by a small increase in Med. *M. galloprovincialis* ancestry in 2015 and 2016 (significant only between 2003 and 2016, $p = 0.0422$). The only other historical sample in our collection was a site in the bay of Brest that showed the absence of dock mussels in 1997 (Pointe de L'Armorique, PtArm97, fig S11). However this site also presented only one introduced individual 20 years

later.

Dock mussels present reduced but residual linkage disequilibria (LD, fig. S23), indicating several generations of recombination since admixture, and this can potentially be used to date the age of the admixture event. We can also note that the genetic map computed from the F2 is coherent with the LD observed in BCs and dock mussels. Using our genetic map in the linkage option of `Structure`, we estimated the admixture time at between 4 and 14 generations, depending on the port considered (table S10 and supplementary methods). Given that mussels reach maturity at ~1 y and the adult lifetime is on average ~10 y, generation time would be around 3-6 years. Therefore the estimated time of admixture is roughly 10 to 85 years ago. We note that the 2003 sample from Cherbourg, which could have been used to calibrate the recombination clock, does not provide a younger time since admixture as we would have expected (on the contrary it is one of the oldest estimate). In addition to the inherent difficulty of this dating and the limitation of our dataset, we note that this estimate assumes neutrality, and no gene flow since admixture. We have evidence, at least in Le Havre, of a constant input of new chromosome tracts from the native *M. edulis*. In addition we can suspect a continuing propagule pressure of Med. *M. galloprovincialis* from the maritime traffic. It is also likely that selection acts to maintain parental gene combinations against recombination (Bierne et al. 2006; Simon et al. 2018). Both effects, gene flow and selection, tend to bias the date estimates towards more recent times. This rough estimate can nonetheless serve as a first indication of a potentially old admixture event (a few decades). A precise estimation of the admixture event will require a precise recombination map in mussels and the distribution of ancestry track lengths along the genome of admixed individuals.

Levels of ancestries of admixed populations Ancestry estimations (Q values, `Structure`, $K = 4$) of each studied population were compared (fig. S22). Both dock mussels and admixed mussels with the Atl. *M. galloprovincialis* differ significantly between sites at the four ancestries considered (Kruskal-Wallis test, $p < 0.001$, except for the Med. ancestry between admixed Atl. *M. galloprovincialis* groups).

Pairwise comparisons for dock mussels showed that only Le Havre have higher levels of Eu. *M. edulis* ancestry compared to other ports (Dwass-Steel-Crichtlow-Fligner test, Benjamini-Yekutieli multiple tests correction, $p < 0.001$) . Le Havre have incidentally lower levels of Med. ancestry ($p < 0.001$ and $p = 0.0715$ for Brest/Havre comparison) and Brest have lower levels compared to Cherbourg and Saint-Malo ($p < 0.001$ and $p = 0.0012$). In terms of Atl. *M. galloprovincialis* ancestry, Brest have higher levels than any other port ($p < 0.001$ and $p = 0.0628$ for Brest/Saint-Malo) while Le Havre have the lowest levels compared to other ports ($p \leq 0.0071$). Finally ports differ slightly in American *M. edulis* ancestry levels. Le Havre has more American *M. edulis* ancestry than Brest, Saint-Nazaire and Saint-Malo ($p \leq 0.0038$). The same pattern is observed between Cherbourg and Saint-Malo ($p = 0.0358$).

Between admixed populations with Atl. *M. galloprovincialis*, the only non-significant pairwise comparisons for the Atl. *M. galloprovincialis* ancestry are GAS/LOF, MCH/HZSB and GAS/JER ($p = 0.0776$). For Eu. *M. edulis* ancestry inside natural admixed populations (ABD, JER, MCH, HZSB), JER has significantly more ancestry from this background than other populations ($p < 0.001$). Likewise ABD has more Eu. *M. edulis* ancestry than MCH ($p < 0.001$) while the HZSB does not differ from ABD and MCH, which is likely due to its large range of ancestry compositions along this natural hybrid zone (fig. 2). The two Norwegian introduced populations (LOF and GAS) do not differ significantly between them among any of the four different ancestry estimations.

Finally, a comparison between several groups of individuals from Brittany was performed to

assess the influence of dock mussels on the local populations. Three groups were chosen: the reference Northern Brittany population (gallo_atl_brit), populations outside the bay of Brest, taken as reference local individuals, and individuals classified as Atl. *M. galloprovincialis* inside the bay (fig. S20). While levels of *M. edulis* ancestry increased and levels of Atl. *M. galloprovincialis* decreased significantly from distant populations to inside the bay of Brest ($p \leq 0.0073$); the levels of Med. *M. galloprovincialis* ancestry did not differ significantly. Nonetheless, we note that the tail of the distribution of individual Med. ancestry is skewed towards higher values (fig. S22). This tail is due to the presence of hybrids between dock mussels and the local native *M. galloprovincialis* (fig. S26).

Observations of other unusual ancestries While the overall genetic composition of many of our sampled populations was as expected, we also obtained some results that are noteworthy in their own right.

First, one population from Korea (KOR, fig. S15) is completely composed of pure Med. *M. galloprovincialis*, corresponding to the known introduction in Asia (McDonald et al. 1991; Han et al. 2017). Another study showed that the introduction on the Pacific coast of the USA was similarly composed by pure Med. *M. galloprovincialis* (Simon et al. in prep.). Those observations exclude the idea that previously observed Med. *M. galloprovincialis* introductions would have been related to dock mussels.

Second, one population in the bay of Biscay, on the Atl. coast of Oléron Island (ROC_VER), presents one individual with pure Med. *M. galloprovincialis* ancestry and a few individuals with some levels of Med. ancestry in an Atl. *M. galloprovincialis* background. Those latter individuals might plausibly be migrants Atl. *M. galloprovincialis* from the Basque Country. Contrary to populations from Brittany, Iberian Atl. *M. galloprovincialis* populations, South to the last hybrid zone with *M. edulis*, have low to medium levels of Med. *M. galloprovincialis* ancestry (see Bilbao port samples, fig. S17 and classification as Atl. in fig. S19). These populations are in contact with the Med. *M. galloprovincialis* background in the South.

Third, the reanalysis of the Coolen (2017) samples did not show any pure *M. galloprovincialis* individuals (fig. S13). However one population contained six individuals composed of 10 to 30% Med. *M. galloprovincialis* ancestry (Q13A, fig. S13). This population is located offshore, at around 25 km from the entry of the port of Rotterdam, the largest commercial port of Europe. Given this greater proportion of migrants at this distance compared to the one observed around other ports, the presence of dock mussels populations in the port of Rotterdam is highly probable and will require further investigation.

Fourth, a few long distance migrants have been observed. For example we found at least one Atl. *M. galloprovincialis* in the port of Le Havre (LeHa_P11, fig. S8). Additionally, two years of mussel collection in 2011 and 2013 on a ferry hull crossing the English channel (i.e., making the connection between a *M. edulis* and a *M. galloprovincialis* region; Plymouth, UK and Roscoff, Brittany, France respectively) showed a mixture of *M. edulis* and Atl. *M. galloprovincialis* individuals (fig. S15, Fer11 and Fer13), highlighting once again the role of ship traffic in the displacement of species and their role as meeting points where hybridisation can occur.

We also detected a signature of Atl. *M. galloprovincialis* in the Northern English Channel and Southern North Sea (Dieppe, Ostende, Ault, Dunkerque "Dun", fig. S5) indicating the presence or recurrent influence of migration in those regions.

Lastly, four other large ports were analysed to search for dock mussels: La Rochelle (Bay of Biscay, France, fig. S16), Bilbao (Bay of Biscay, Spain, fig. S17) and New-York city (USA, fig. S18). None of those ports showed the presence of admixed *M. galloprovincialis* dock mussels. Interestingly the port of New-York showed higher levels of South-Eu. *M. edulis*

ancestry, up to 30%, compared to other populations from Long Island Sound (fig. S18). Therefore we cannot exclude the possibility that there has been an introduction of Eu. *M. edulis* in or close to the port of New-York.

3.2 Geographic clines show sharp and concordant transitions at the sea-port interface

We fitted geographic clines of allele frequencies for five transects: two independent transects in Le Havre for the North and the South basins of the port, and one in each of the other ports excluding Saint-Malo (which did not have enough sampled sites). Pure *M. edulis* individuals were removed for the analysis in the bay of Brest and Atl. *M. galloprovincialis* individuals for the ports of Le Havre, Saint-Nazaire and Cherbourg. Transects start at the inner-most population of each port (reversed triangles fig. 3) and least-cost distances from those points were used to approximate distances between sites and project them to a unique dimension. In the case of the bay of Brest the inner-most population is taken to be the upstream point in the Élorn estuary. Only differentiated loci are presented in figure 4 (panel A-D: allele frequency difference (AFD) > 0.5, panel E: AFD > 0.3; see fig. S27-S31 for details).

Allele frequencies shift sharply at the entrance of ports (fig. 4A-D) and clines are highly concordant both between markers and with the mean ancestry cline (red line). Compared to the reference Med. *M. galloprovincialis* frequencies, dock mussels show a global decrease of allele frequency due to a genome wide introgression from the local species.

Clines have narrow average widths across all ports. Average widths are 3.99 km ($SD = 1.80$) and 1.30 km ($SD = 0.52$) for the North and South transects of Le Havre respectively (fig. 4A-D); 7.37 km ($SD = 5.38$) in Cherbourg (fig. 4C); 2.16 km ($SD = 2.15$) in Saint-Nazaire (fig. 4D), and 18.51 km ($SD = 14.03$) in the bay of Brest (fig. 4E).

The difference between the North and South transects in Le Havre is best explained by the presence of more *M. edulis* or hybrid individuals at the entry of the North basin (fig. 3A). The interpretation in the bay of Brest is more difficult due to two factors. First, the spread of the introduced background and sympatry with local mussels in several populations (fig. 3E-F) make allele frequencies more variable between close populations. Second, we used a reduced number of differentiated markers between Atl. and Med. *M. galloprovincialis* in our dataset and their level of differentiation is globally lower.

3.3 Repeatability of allele frequency deviations between admixture events

If admixture events are non-independent (e.g., due to migration between ports), or if admixture events are independent, but lead to repeatable patterns of natural selection, then we would expect to see the same alleles over- or under-represented in different locations.

We cannot compare allele frequencies directly, because different locations are characterised by different overall levels of ancestry. Therefore, for each marker, in each location, we calculated its deviation from expected values. These expected frequencies were calculated from the allele's frequency in the reference parental populations, combined with the overall levels of ancestry in the sampled location (this is Barton's concordance analysis, eq. 1-3).

Examination of these allele frequency deviations showed some suggestive similarities between admixture events. For example, the mitochondrial marker (601) is differentiated between the Med. *M. galloprovincialis* and the Atl. lineages (fig. S37). This locus exhibits large distortions

(D) towards the Med. lineage in Le Havre, Cherbourg and Saint-Nazaire (0.11, 0.16, 0.13 respectively), while displaying smaller distortions in Brest and Saint-Malo (0.03 in both cases).

More formally, the repeatability of admixture events can be assessed by correlating the complete set of allele-frequency deviations between events. Figure 5 shows Pearson correlation coefficients between deviations for pairs of events. The significance of these correlations was assessed after accounting for multiple testing and potential non-independence.

Figure 5 shows results for four types of comparison. Panel (i) shows results for pairs of admixture events involving the same parental backgrounds in similar conditions. In particular, we examined all pairwise comparisons between the five introductions to French ports ("int_med/int_med"), the two Norwegian introductions ("int_atl/int_atl"), and the natural hybrid zones ("nat_atl/nat_atl"). In each case, the allele frequency deviations are significantly positively correlated between events, with medium to large effect sizes (fig. 5 and fig. S32-S33). The same was also true when we compared the Norwegian introductions to the natural hybrid zones involving the same genetic backgrounds (Atl. *M. galloprovincialis* and Eu. *M. edulis*). This is shown in panel (ii) of Figure 5A, labelled "int_atl/nat_atl".

Remarkably, strong correlations were also observed when we compared the French port introductions to lab crosses involving the same lineages (Med. *M. galloprovincialis* and South-Eu. *M. edulis*). Results, shown in panel (iii) of Figure 5A, show that the correlations were strongest for lab backcrosses (labelled BCs), and much weaker, and non-significant for the F2. This is consistent with the genetic makeup of the dock mussels, which are closer to BC genotypes than to F2 (fig 2).

Panels (i)-(iii) indicate that admixture events of very different kinds can lead to strongly repeatable results. But this is only true when the same genetic backgrounds are involved. To show this, Figure 5A panel (iv) shows results from pairs of admixture events involving different backgrounds (e.g. Med. *M. galloprovincialis*/*M. edulis* vs. Atl. *M. galloprovincialis*/*M. edulis*). In this case, effect sizes are small to medium, and sometimes negative.

4 Discussion

We uncovered introductions of Med. mussels in five French ports of Western France, confirming previous observations made once in Cherbourg in 2003. These dock mussels display high similarity between ports, and are all admixed populations. Admixture occurred with the genetic lineages encountered locally. In addition, our spatial sampling in ports allowed us to document striking localisation of non-native genotypes to the interior of the ports. At each location, we observed narrow shifts at the sea-port interface. Some variation was, however, observed between ports, potentially due to their different layouts and conditions. Finally, by including and reanalysing *M. galloprovincialis* populations in Norway, experimental crosses, and newly identified admixed populations from several sites, we were able to compare admixture patterns between equivalent situations as well as between different genetic backgrounds and thus investigate the extent of parallelism in the secondary admixture processes.

4.1 The introduction of dock mussels

The analysis of a sample from 2003 in Cherbourg revealed the presence of the Med. *M. galloprovincialis* genetic background, a non-native taxon in the study area, and its admixed nature (Simon et al. in prep.). Our investigations have uncovered the extent, both inside the port of Cherbourg, and in other ports, of those introduced mussels and their relative homogeneity given the possibilities of admixture with two different lineages of the *Mytilus* complex. Dock

mussels constitute homogeneous populations composed of around 70% Med. *M. galloprovincialis* ancestry.

The evidence of limited natural dispersal outside ports, presented in this study, provides a strong case for a saltatory colonisation of ports through human-mediated "jump dispersal". In the bay of Brest, the presence of dock mussels with similar genetic compositions to the other ports, (see the similarity of ancestry distributions across docks mussels in all ports, fig. S22) where the local native species is however different (i.e., predominantly Atl. *M. galloprovincialis* rather than *M. edulis*), suggests that the admixture with *M. edulis* happened before the introduction of these dock mussels in this bay. Despite the fact that a few *M. edulis* have been found in this area, because of aquaculture of imported spat from the Bay of Biscay, the most parsimonious hypothesis is that dock mussels arrived already admixed in the port of Brest. Ship traffic is thus likely to be the main source of this introduction to ports. The five studied infrastructures are large commercial and/or military ports that may have facilitated the primary introduction of mussels (Hewitt et al. 2009; Sylvester et al. 2011). Given the presence of marinas in the vicinity of the large studied ports and their colonisation by dock mussels, they constitute a possible way of secondary expansion at a regional scale. Indeed, marinas and associated activities, e.g. leisure boating, have been shown to contribute to regional NIS expansion (Clarke Murray et al. 2011) and create chaotic genetic structure in both native and non-native species inhabiting these artificial habitats (Hudson et al. 2016; Guzinski et al. 2018). For now, in the bay of Brest, only the marinas closed to the large port contained dock mussels. The other marinas outside of the bay (e.g. Camaret and Morgat, fig. S11 Brest-11 and 13 respectively) – potentially exchanging a lot of traffic with Brest marinas – did not, and this supports the absence of a secondary introduction. Dispersal seems therefore so far limited to large port infrastructure, and nearby marinas, with dispersal due to large vessel traffic. This situation might however change over time, and genetic monitoring should be pursued.

Interestingly, in 1978, Prof. David Skibinski analysed hybrids from natural populations in the Swansea region (UK) with allozymes (Skibinski et al. 1978) and noticed that the "King's dock" populations (Swansea port) were unusual (fig. S38). Those populations showed linkage and Hardy-Weinberg equilibrium and intermediate allele frequencies between *M. edulis* and *M. galloprovincialis*. A closer look at the allele frequency shows that, at one particular allozyme subsequently shown to differentiate Atl. from Med. *M. galloprovincialis* (*Ap*, Quesada et al. 1995b), King's dock populations have allele frequencies that are closer to those of Med. mussels than to local Atl. *M. galloprovincialis*. This evidence suggests that introduced dock mussels were already present, and already admixed with *M. edulis* at the same level in the Swansea port, 40 years ago. The term dock mussels has been chosen in reference to this work. We do not know if dock mussels persisted in the Swansea port and this matter needs further investigation.

Recently, Zbawicka et al. (2018) reported the presence of an admixed population between introduced Med. *M. galloprovincialis* and native *M. platensis* close to the city of Puerto Madryn in the middle of the Atlantic Argentinian coast. Their randomly ascertained SNPs did not allow a precise analysis of individual admixture proportions but the average admixture appeared well balanced. In this issue, Popovic et al. report two independent introductions of *M. galloprovincialis* in Australia, one by the Atl. lineage in Batemans Bay and the other by the Med. lineage in Sydney Harbour, both accompanied by admixture with the native genetic background (*M. planulatus*). In New-Zealand, Gardner et al. (2016) report admixture between the introduced *M. galloprovincialis* and the local *Mytilus* species. Such reports are additional indications of the frequent occurrence of the admixture process where *M. galloprovincialis* has been introduced in an area already inhabited by a native lineage of *Mytilus*. Admixture did

not happen during the introduction of Atl. *M. galloprovincialis* in South Africa formerly free of *Mytilus* mussels (Grant and Cherry 1985), nor during the introduction of Med. *M. galloprovincialis* in California (Saarman and Pogson 2015) and Asia (Korean sample in this study). The points of introduction of the latter two cases were likely devoid of dense populations of native mussels. Events of admixture are not restricted to *M. galloprovincialis*, for instance all species of the *Mytilus* complex have been shown to have influenced the Kerguelen Islands mussel populations (Fraïsse et al. 2018a).

In addition to allowing the study of introduction and evolutionary biology, this model could be of interest for the recent field of urban ecology and evolution, investigating the impact of urbanisation on evolutionary trajectories and the feedbacks with the environment (Rivkin et al. 2018; Thompson et al. 2018). The marine environment is not left untouched by urbanisation and human infrastructures have large impacts on coastal communities and their abiotic conditions (Mayer-Pinto et al. 2018; Critchley and Bishop 2019). This is the "Ocean Sprawl", in the words of Duarte et al. (2012), which has broad effects (see for a review Firth et al. 2016). Human activities impacts includes both connectivity modifications and environmental and toxicological changes.

4.2 Confinement of the introduced mussels, introgression and potential impacts

In all studied ports, the introduced dock mussels form sharp human-induced hybrid zones at the sea-port interface. By contrast, natural clines in mussels are usually on the order of tens to hundreds of kilometres (Lassen and Turano 1978; Väinolä and Hvilsom 1991; Strelkov et al. 2017). If the natural clines are due to post-zygotic selection in a tension zone model (Barton and Hewitt 1985; Bierne et al. 2002a), then the narrow clines in ports imply additional processes. Those processes could include habitat choice by mussels during the larval settlement stage at a small spatial scale (Comesaña and Sanjuan 1997; Bierne et al. 2003; Katolikova et al. 2016) or early stage post-settlement ecological selection to the port environment for example through attachment strength (Willis and Skibinski 1992), pollution tolerance (Loria et al. 2019 for a review and McKenzie et al. 2011 for an example in a bryozoan), or competition for space linked to different growth rates (Branch and Steffani 2004; Saarman and Pogson 2015). For example, genetic differentiation in mussels has been shown to be associated with sewage treatment plants (Larsson et al. 2016; Larsson 2017).

Although our sampling around ports was not exhaustive, dock mussels do appear to be restricted to the port interiors, with only a few migrant introduced mussels detected in distant populations. While the presence of introduced migrants up to 30 km from ports may appear concerning, most distant individuals are hybrids between dock mussels and the local background (fig. 3 and S25-S26). Therefore, we can hypothesise that the propagule pressure from ports will be swamped by large native populations for most of the ports. Conversely, native mussels are relatively rare inside the ports (except for Brest). Were they more numerous, hybridisation might favour an increase of genetic pollution by the possibility of backcrossing to the native mussels. The concern of genetic pollution seems increased in the bay of Brest where the potential for dispersion and hybridisation appears greater. Additionally, populations of introduced mussels were found in basins closed by locks (Saint-Malo, Le Havre, Cherbourg, Saint-Nazaire). In such contexts, both the exit and entry of mussel larva from any species may be limited. Those populations may act as reservoirs of introduced backgrounds.

Introduced dock mussels agree well with the models and review of Currat et al. (2008). They show that introgression from the local species to the introduced one is to be expected,

and that it can reach high levels, both in cases with and without competition between the two species. Additionally, their models show that the hypothesis of adaptive introgression is not required, and that purely neutral processes are consistent with large-scale asymmetrical introgressions from the native to the introduced species. They conclude that genetic pollution by NIS is unlikely to be substantial. However when the invasion wave is halted and trapped at a natural barrier, density trough, or ecotone, introgression can start to proceed in both directions. Introgression of native mussel populations by dock mussel alleles could therefore become a concern. For example, in the westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), introgression impacts the fitness of native populations (Muhlfeld et al. 2009) but selection against introduced alleles in wild populations seems to be acting (Kovach et al. 2016). However in the brown trout (*Salmo trutta*), an haplotype-based method showed that residual introduced tracts are present in native populations and go undetected by classical ancestry estimation methods (Leitwein et al. 2018).

The bay of Brest presents an interesting case study both in terms of implicated species – this is a crossroad between three lineages – and of introduction. In this area, unlike the other ports, introduced mussels were found far beyond the human-made structures. Nonetheless, even in sites that are distant from ports, mussels were predominantly found on artificial structures (buoys, pillars, piers, etc.). However this observation may be more related to space competition with oysters on natural sites than to habitat selection as finding any genetic background in the the bay on rock substrates was difficult.

The spread of dock mussels in the bay of Brest might be due to several interacting factors. First, the port – and notably the commercial area – has a more open layout compared to the other four ports (some of which, such as Saint-Nazaire, have locks at their entry). Second, compared to other ports, habitats suitable for dock mussels might have been available. Third, the closer genetic distance with Atl. *M. galloprovincialis* when compared to *M. edulis* might facilitate hybridisation by avoiding stronger reproductive incompatibilities (both pre- and post-zygotic).

When interacting species have accumulated too many incompatibilities for hybridisation to lead to viable and fertile offspring, inter-specific mating represents lost reproductive effort (Allendorf et al. 2001). For less reproductively isolated species, hybridisation has been considered by Mesgaran et al. (2016) as a way to escape demographic Allee effects during colonisation. Given that mussel populations suffer from a strong Allee effect, hybridisation has potentially provided the initial demographic boost to the first introduction of Med. *M. galloprovincialis*. Conversely, hybrid breakdown would have impeded both the introduction of the hybrid background, which would then have required a tremendous propagule pressure from maritime traffic. The same applies to the subsequent spread of background, even if fitter (Barton and Turelli 2011), and this could explain its confinement inside the ports. Stochasticity (drift and variation in population density and dispersal) can free the introduced background after a lag time (Piálek and Barton 1997). Although the delay is expected to be long, confined dock mussel populations could represent hidden bombshells able to escape and spread globally in the future.

The local impact of introduced dock mussels is hard to predict and will require further investigation. *M. trossulus* has been introduced and has produced large losses to aquaculture in Loch Etive in Scotland due to their colonisation of culture ropes and their shell being more fragile (Beaumont et al. 2008; Dias et al. 2009). In Brittany and Normandy, most of the cultured mussels are imported spat from the bay of Biscay because *M. edulis* is easier to cultivate, with a shorter reproduction period, and has a higher commercial value for consumers than Atl. *M. galloprovincialis*. Therefore, spat collection of introduced mussels and involuntary

culture of the wrong genetic background should impact the quality of cultured mussels and the growing cycle used in mussels farms, but only in case of a massive invasion. Michalek et al. (2016) report impacts of hybridisation on *Mytilus* aquaculture in Scotland and Larraín et al. (2017) raise concerns of economic impacts in Chile. Nonetheless we are left with the fact that in ports and in natural environments in the bay of Brest, dock mussels have probably displaced the native lineages.

While there may be few direct impacts of dock mussels on native and aquaculture populations, we should be more concerned about the potential parasites they may have brought into natural and cultivated populations, or about a spillover effect if they prove more resistant to native species pathogens. Parasite hitch-hiking during introduction and their transmission to native species has been documented both in terrestrial and marine systems (Torchin et al. 2002; Prenter et al. 2004). This event is made even more probable by the fact that we are dealing with species from the same complex and that can live in sympatry. This issue will require further investigation. We can note however that we did not detect the *M. trossulus* transmissible cancer (Metzger et al. 2016; Riquet et al. 2017) in dock mussels.

This fine-scale confinement within ports raises new questions about the spread of plankton-dispersing invaders in the sea. To understand the introduction process in the different ports, there needs to be an integration of genetics and ecology (Lawson Handley et al. 2011). However, we have a large gap in our ecological knowledge of the port environments and what influences mussel populations. A thorough study of the ecology of mussels in ports will be needed to untangle the roles of ecological variation in the distribution of dock mussels. Both habitat choice and post-settlement selection are likely to play a role. Additionally, modelling of larval dispersal at a really fine scale in ports is lacking. However Jouanneau et al. (2013) show in a physical study of Boulogne-sur-Mer (France) harbour the formation of eddies and particle retention, which are highly dependent on physical constraints (wind, tide) and on harbour design. All of those parameters also apply to the ports studied here, and are highly important for gene flow between the introduced and native backgrounds. Similarly, Guillou and Chapalain (2012) developed a model to estimate tide-influenced waves in Le Havre port. Oceanographic modelling studies are needed to understand the potential for NIS spread from ports. Such modelling studies would require an accurate and fine scale description of habitats. For now, at a larger scale, we could take advantage of existing models where integration of a biological module has been performed. For instance, in the bay of Brest, the model of Bessin (2017) could help investigate the relative weights of dispersion, habitat selection and ecological constraints on the distribution of genetic backgrounds.

4.3 Parallelism of distortions

The parallelism in allele frequency distortions that we observed between admixture events, suggests that such events can be highly repeatable. This is probably due to a combination of processes. As discussed above, port introductions are expected to partly share a pre-introduction common history of admixture. The two introduced Atl. *M. galloprovincialis* populations we studied in Norway are also likely to share the same history of admixture. However, the composition in *M. edulis* ancestry of these populations is in accordance with an independent admixture event with the local *M. edulis* background. Naturally admixed Atl. *M. galloprovincialis* combine an old history of introgression (Roux et al. 2014; Fraïsse et al. 2018b) during glacial oscillation periods with ongoing local introgression from the native *M. edulis* populations in direct contact within the mosaic hybrid zone observed today (Fraïsse et al. 2016; Simon et al. in prep.).

Shared colonisation history cannot be the whole story, however, because we also found repeatable patterns between admixture events that must be considered independent. This includes not only the comparisons of natural admixture to the introduced Atl. *M. galloprovincialis* (involving two different backgrounds of *M. edulis*: South and North European), but also the comparison of port samples to experimental backcrosses.

Our comparison of Atl. *M. galloprovincialis* admixtures includes populations with a wide variety of contributions of the parental proportions. These range from a high *M. edulis* contribution in JER to a high Atl. *M. galloprovincialis* contribution in MCH. The high positive correlations of distortions observed between all Atl. *M. galloprovincialis* admixture, despite variable contributions of the two parental backgrounds, is particularly interesting. The calculation of D corrects for ancient introgression of parental backgrounds, and we are unlikely to have missed a hidden parental population given our broad geographic survey (Simon et al. in prep.) and the large-scale genetic panmixia usually observed in mussels outside hybrid zones (e.g. East vs. West Mediterranean Sea). Genomic regions do tend to consistently deviate toward an excess of *M. galloprovincialis* ancestry or an excess of *M. edulis* ancestry. This suggests selective processes and a shared architecture of the barrier to gene flow. A first possible explanation is that some loci are closer to barrier loci than others (Ravinet et al. 2017). Barrier loci can be local adaptation genes or genetic incompatibilities. Schumer et al. (2018) found that in several events of admixtures between swordtail fish species contributing differently to the resulting population, local ancestry were nonetheless positively correlated. They showed that parallel correlations, despite opposite parental contributions, can be the result of selection in the same direction to resolve pairwise epistatic incompatibilities. We offer the same interpretation for mussels. *M. edulis* and *M. galloprovincialis* are found at the end of the speciation continuum (Roux et al. 2016) and are expected to be incompatible at a high number of substitutions (Simon et al. 2018). With such a highly polygenic determinism of post-zygotic selection one expect a correlation between recombination rates and introgression (Barton and Bengtssont 1986) which has recently been observed in a number of study system (*Mimulus* Aeschbacher et al. 2017, sea bass Duranton et al. 2018, oyster Gagnaire et al. 2018, stickleback Roesti et al. 2013, swordtail fish Schumer et al. 2018 or *Heliconius* Martin et al. 2019). An interesting interpretation of the parallelism observed would be that our loci belong to genomic regions with different rates of recombination.

Equally notable to the strongly repeatable patterns when the same *M. galloprovincialis* lineages are involved is their absence when different lineages are implicated. A possible explanation is that different sets of incompatible loci may be implicated in the reproductive isolation between *M. edulis* and the two *M. galloprovincialis* lineages. However, the history of divergence between the two *M. galloprovincialis* lineages is much younger than the divergence with *M. edulis* and most of the fixed mutations are expected to be shared by the two lineages (Fraïsse et al. 2016). Additionally, Atl. *M. galloprovincialis* is in contact with *M. edulis* while Med. *M. galloprovincialis* is not. Atl. *M. galloprovincialis* has experienced a punctuated history of introgression possibly swamped by bi-stable incompatibilities with an asymmetric advantage to the *M. edulis* allele (Gosset and Bierne 2013; Fraïsse et al. 2016; Simon et al. in prep.). This differential introgression might have erased, or even reversed, the selective effects in the two *M. galloprovincialis* backgrounds. This hypothesis requires further theoretical and experimental investigation. Finally, given that karyotypic differences have been demonstrated between the two *M. galloprovincialis* lineages (Martínez-Lage et al. 1996), they potentially exhibit different recombination landscapes impacting the outcome of distortions.

Mytilus mussels, with their introduction and hybridisation potentials, are a particularly useful model to study the parallelism of admixture events and more generally the outcome of introductions with hybridisation. Our study shows that admixture between the same genetic backgrounds are highly repeatable. This repeatability can be explained both by a shared history of pre-introduction admixture and parallel genomic processes. One of the anthropogenic hybridisation case, the dock mussels, has been thoroughly investigated in term of spatial distribution of ancestries between the natural habitats and the ports. We still have a lot to learn from those introduction events in terms of ecology and genomics. Follow up investigations will be needed to understand how selection, hybridisation, environmental conditions and dispersal are shaping the distribution and genomic architecture of those dock mussels and similar introductions.

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Figures

Figure 1: **(A-B)** Principal Component Analysis of reference samples and studied groups: **ports** - port sampling sites (inside the five French ports or really close), 500/1930 individuals were drawn at random for visual clarity; **int_atl** - introduced Atlantic *M. galloprovincialis* (Lofoten LOF and Gåseid GAS); **nat_atl** - naturally admixed Atlantic *M. galloprovincialis* from hybrid zones in Scotland (ABD), the Norwegian Sea (MCH), South Brittany (HZSB) or Normandy (JER); **crosses** - laboratory crosses (F1, F2, BCF1 and BCG) between South European *M. edulis* (edu_eu_south) and West Mediterranean *M. galloprovincialis* (gallo_med_west). *M. trossulus* samples were not considered. **(C)** Schematic tree of lineage relationships presenting group names and colour schemes. External circle colours represent known local introgression from another lineage.

Figure 2: Location and ancestry composition of sites for reference populations and admixture events. Each barplot represents ancestry per individual of the focal site determined by `Structure` with $K = 4$. In all barplots, individuals have been sorted by their level of Mediterranean *M. galloprovincialis* ancestry. Coloured coastlines indicate the approximate distribution of considered genetic backgrounds. Hybrid zones are coloured in purple. Points A to F correspond to the ports of Le Havre, Cherbourg, Saint-Malo, Saint-Nazaire and Brest respectively, which are detailed in fig. 3.

Figure 3: Ancestry composition of sites for each port. Barplots represent the same information as in fig. 2. Barplots at the map edges correspond to distant populations with the least cost path distance from the port indicated in parentheses. The inner-most populations used to fit geographic clines are indicated by the reversed triangles. **(A)** Le Havre; notice the two different main basins, one North and one South, used in following geographic cline analyses; the arrow indicate a population located on the estuary side of the dyke, characterised by a preponderance of *M. edulis* individuals. **(B)** Cherbourg; dates indicate collection date; where collection dates are missing it corresponds to 2017. **(C)** Saint-Malo. **(D)** Saint-Nazaire. **(E)** Bay of Brest; map **(F)** corresponds to the inset rectangle in this map.

Figure 4: Geographic clines computed with the package `hzar`. The x-axis is the distance from the most inward point in ports indicated by the reversed triangles in figure 3 and determined by a least-cost path analysis. Top crosses indicate the distance of each site considered. For representation purposes, some distant points are not displayed, but were used in the cline fit. Only alleles with a frequency difference of 0.5 between left-most port population and sea-side reference are presented (except for panel **E** where the threshold is 0.3). Coloured segments make the correspondence between the local fit and reference frequencies. For A to D, references are Mediterranean *M. galloprovincialis* on the left and *M. edulis* on the right. For E, the right hand side reference is the local Atlantic *M. galloprovincialis*. The red cline is the mean cline computed from the Mediterranean *M. galloprovincialis* Q-value from `Structure`, in mean proportion of ancestry. The cline of the female mitochondrial marker (601) is highlighted in green when present. **(A)** Le Havre, North transect. **(B)** Le Havre, South transect. **(C)** Cherbourg. **(D)** Saint-Nazaire. **(E)** Bay of Brest.

Figure 5: **(A)** Pearson's r correlation coefficients of distortions (D) between groups of admixture types. Each point is a correlation between two sites (e.g. havre *vs.* cher or BCF1 *vs.* MCH). The significance level correspond to the combination of p values among

comparisons (see methods). int_med - introduced Mediterranean i.e. dock mussels; int_atl - introduced Atlantic i.e. Norway admixed populations; nat_atl - naturally admixed Atlantic *M. galloprovincialis* i.e. ADB, JER, MCH and HZSB. Four types of comparisons were tested: (i) intra - comparisons among the same types of admixture events; (ii) inter_atl - comparison between *M. edulis* (two different backgrounds) and Atlantic *M. galloprovincialis* admixture; (iii) inter_med - comparisons between admixture events involving South European *M. edulis* and Mediterranean *M. galloprovincialis*; (iv) inter_lineages - comparisons of admixture events between different backgrounds. **(B-G)** Examples of correlations between distortions. All correlations presented are significant and linear models with 95% confidence intervals are plotted. The colour of the axis shows the direction of the distortion in term of lineage. Pies show the mean ancestry composition of the population considered. Distortion corresponding to the mitochondrial marker (601) is highlighted in green in panels B-G.

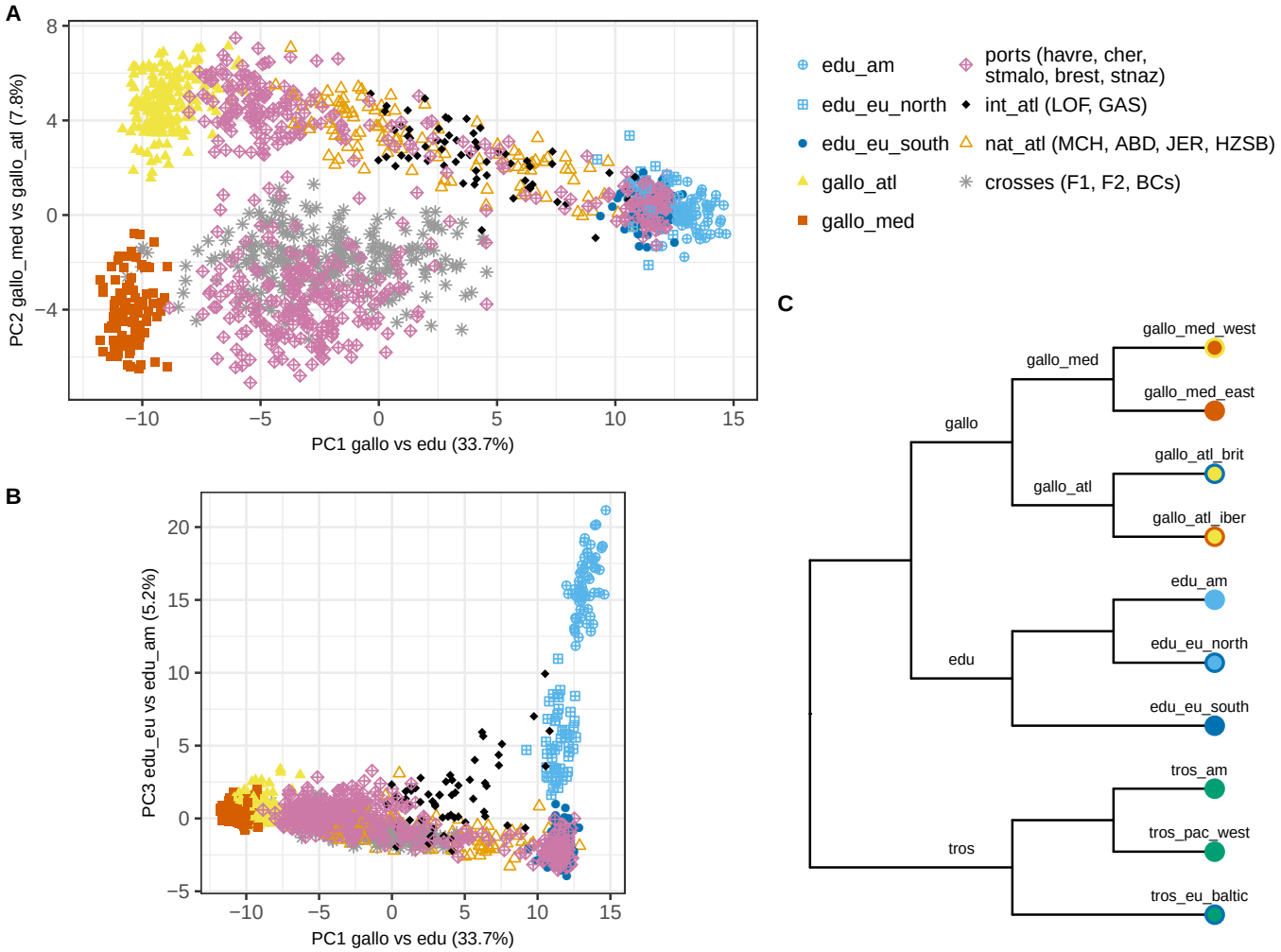

Figure 1: Principal Component Analysis

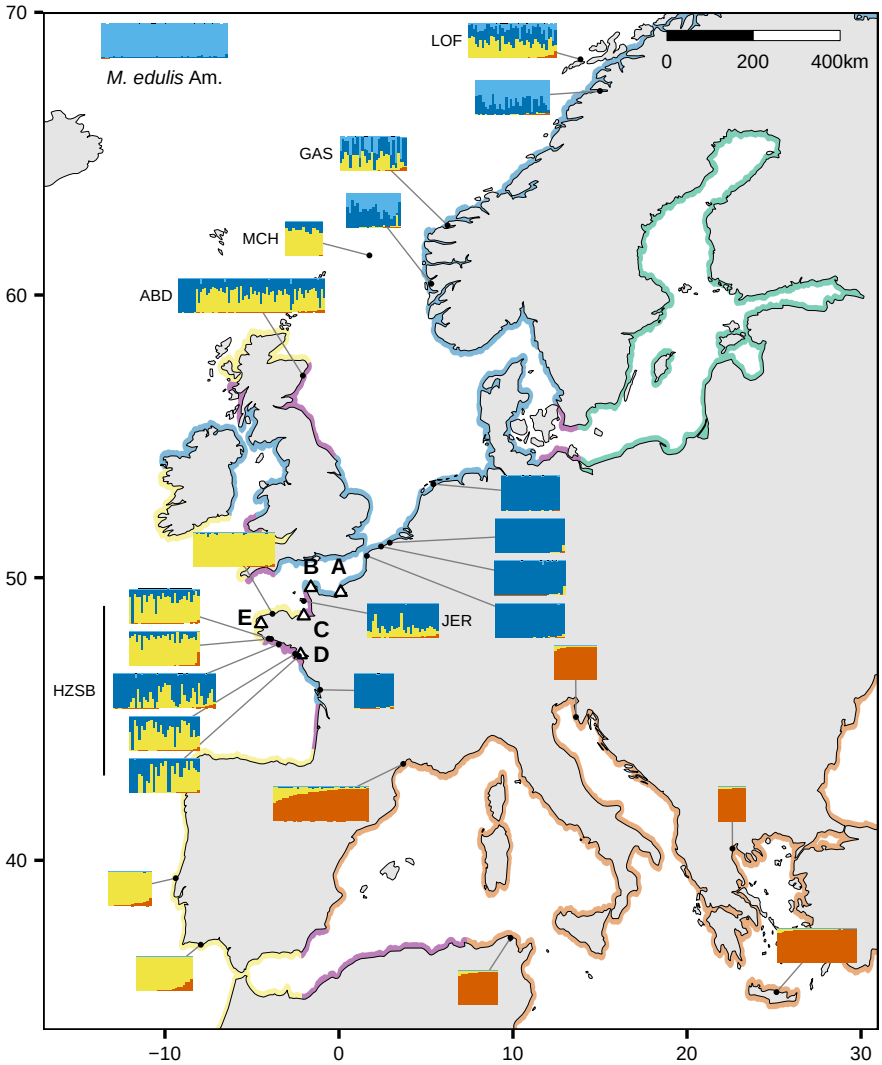

Figure 2: Map for principal samples and references

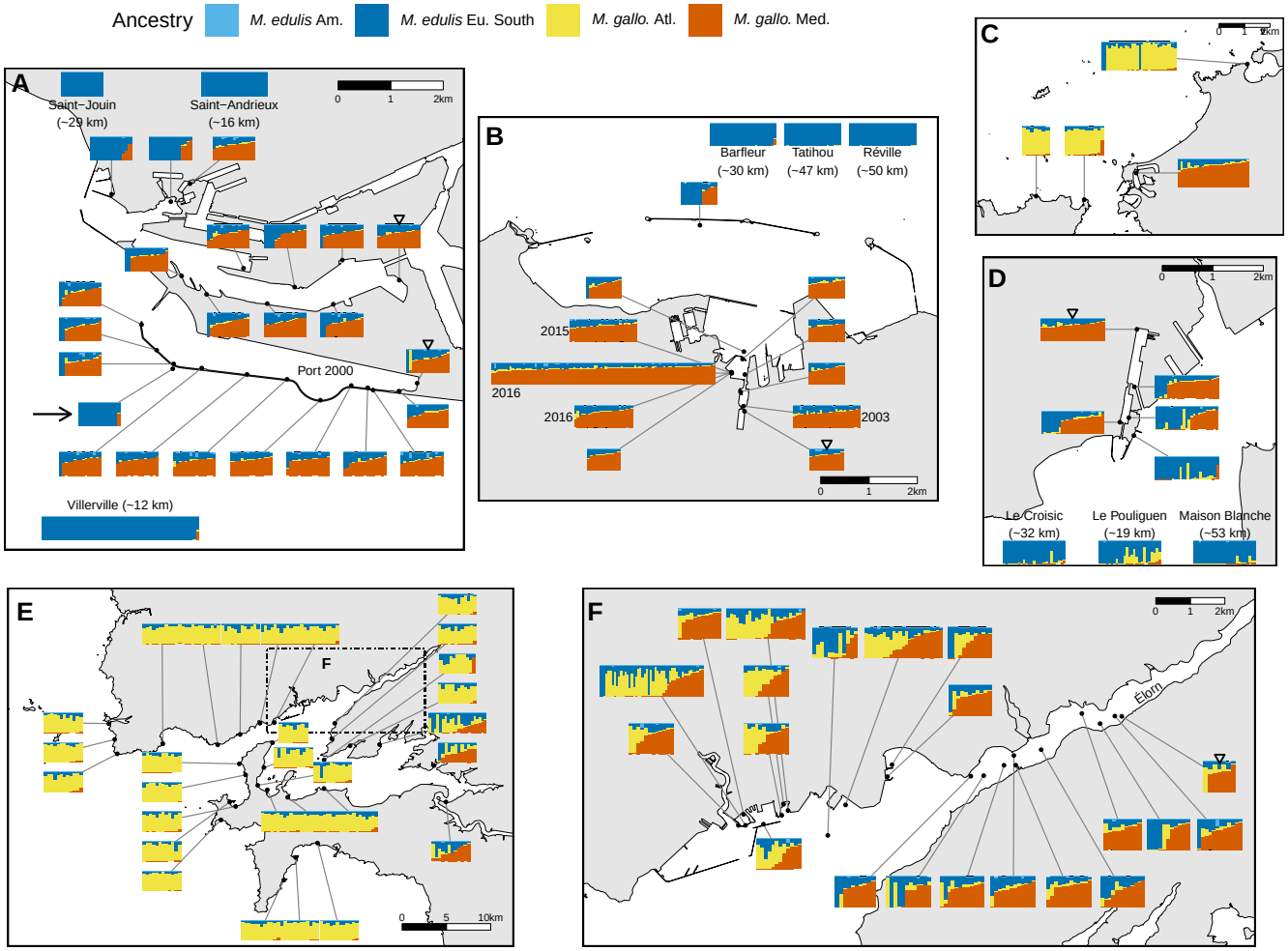

Figure 3: Ancestry composition of sites for references and each port.

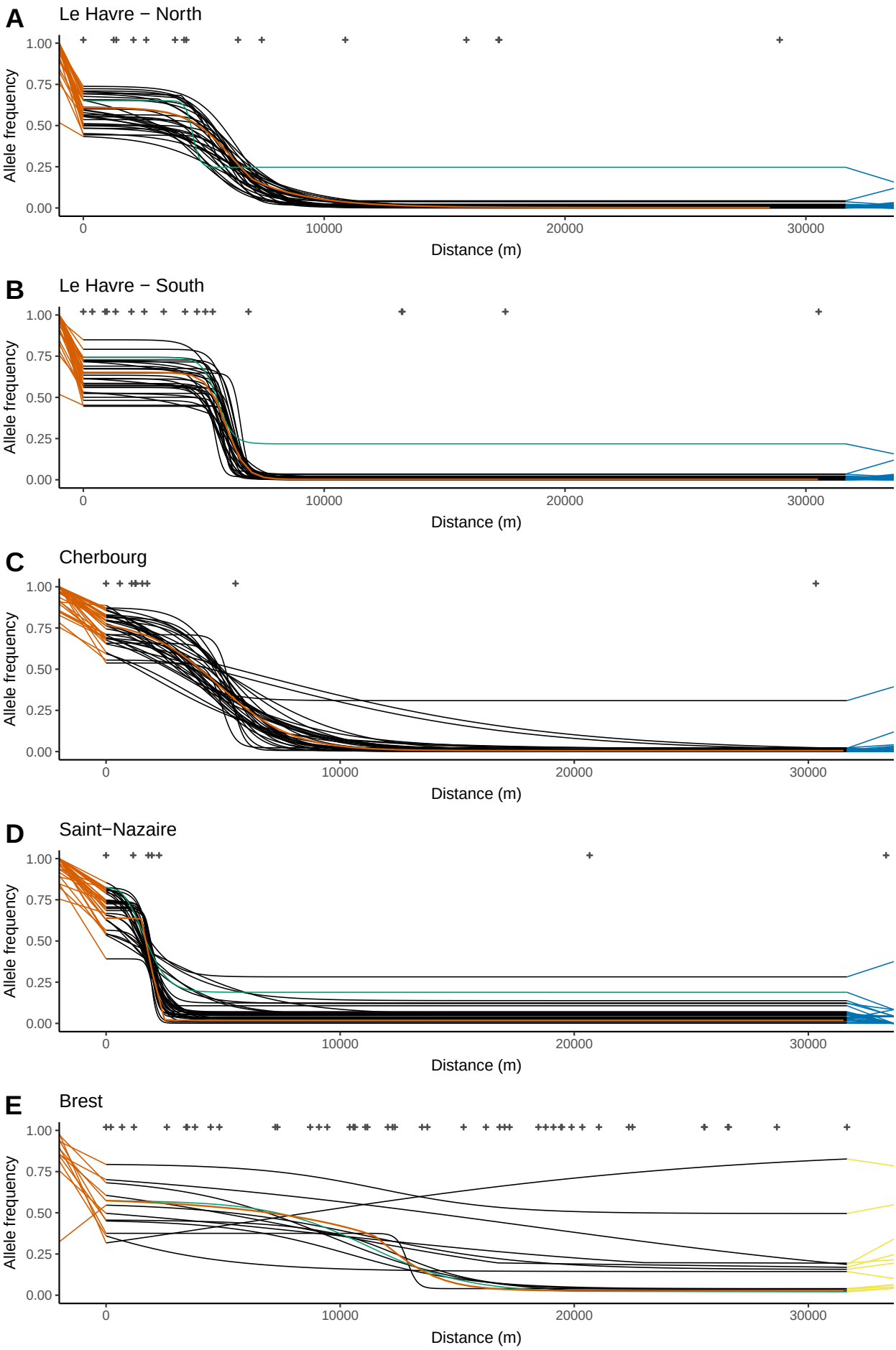

Figure 4: Geographic clines.

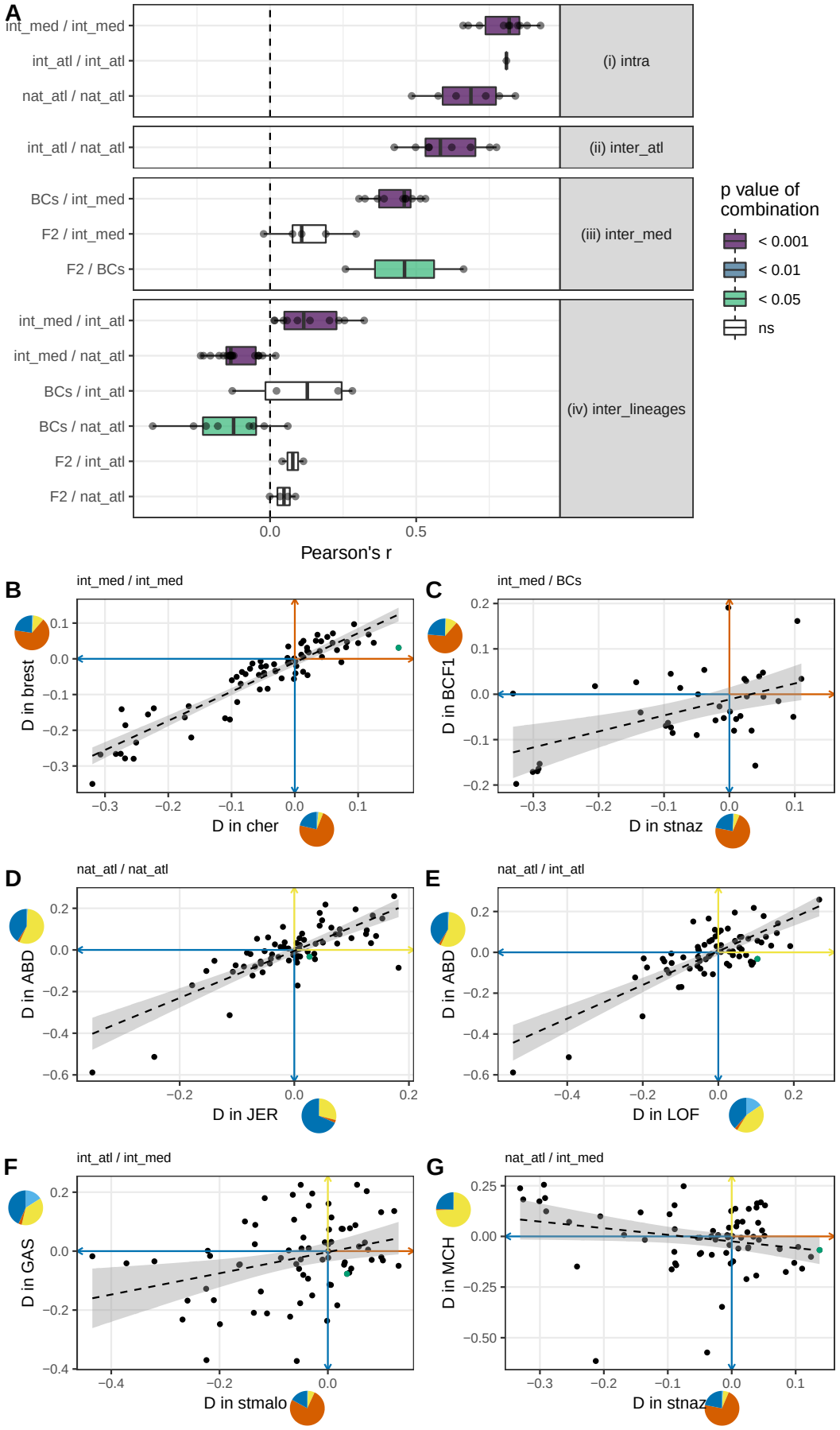

Figure 5: Correlation of frequency distortions and combination of p values