Appendices of the paper entitled:

**Anthropogenic transport of species across native ranges: unpredictable genetic and evolutionary consequences**

by

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**Appendix S1. Studied species**

The vase tunicate, *Ciona intestinalis* [1] (Ascidiacea, Tunicata, Chordata), is a solitary hermaphroditic ascidian that inhabits shallow waters from temperate to boreal regions [2]. Populations of *C. intestinalis* inhabiting natural habitats are poorly known [3] and as such the vast majority of studies are conducted using individuals from marinas and ports. Its chordate body plan and high tolerance to anthropogenic conditions makes this species an ideal model organism for studies in developmental biology and evolutionary genomics [2, 4-6]. Comparisons of genomic DNA from northern European and Pacific *C. intestinalis* individuals revealed the putative existence of cryptic diversity [7]. Recent morphological analysis has determined though that the most widespread types (*C. intestinalis* type A and B) are distinguishable by several morphological characteristics (e.g. the presence or absence of tubercular prominences on the tunic of the siphons) [8, 9]. These studies concluded that *C. intestinalis* type A corresponds to *Ciona robusta* and *C. intestinalis* type B to *Ciona intestinalis* *sensu* Millar 1953[8-10]*.* *Ciona robusta* is thought to be native to the north-western Pacific Ocean [11], although it has been introduced to the eastern Pacific Ocean [12, 13], Europe [2, 11, 14] and the southern hemisphere [15, 16]. *Ciona intestinalis* is believed to be native to the northern Atlantic Ocean and has since presumably been introduced to the western Atlantic coastlines [11]. In the English Channel, *C. robusta* has been introduced within the native range of *C. intestinalis*, leading to an area of sustainable sympatry [14, 17]. When invasive, *C. intestinalis* and *C. robusta* can cause serious economic damages to aquaculture and ecological changes due to negative effects on native assemblages [18-21].

*Ciona intestinalis* produce motile, lecithotrophic larvae that remain in the plankton for short periods of time before settlement [22]. This leads to a limited dispersal capability, with models predicting a maximum dispersal of *c.* 6 km [23-25]. Therefore, long-distance dispersal of *C. intestinalis* among harbours or marinas separated by 10s of Km, where natural habitats are not available in between, must be the result of human-mediated transport. The disparity in dispersal distances between natural and artificial transport for *C. intestinalis* allows the two dispersal types to be easily discerned.

**Appendix S2.****Details on sample collection**

We sampled *C. intestinalis* from fifteen sites along the English Channel between June and December 2014 (Table 1, Fig. 1). These sites were selected as they were either not previously sampled [i.e. Town Quay, National Oceanography Centre Southampton (NOCS), Isle of Wight, Jersey], included in temporal monitoring and sampling (i.e. South English Channel populations [17]) or had not been sampled since 2007 [14]. All sample sites were artificial habitats. One marina was sampled at each geographic location, with the exception of Jersey where two neighbouring marinas (St. Helier Marina and Elizabeth Marina in Jersey; Town Quay and NOCS in Southampton) were sampled. Roscoff-Bloscon is visited by Ferry (regular Plymouth-Roscoff line), professional fishing boats and recreational vessels, whereas the other marinas are visited by recreational vessels. The two marinas in Jersey as well as the Bas-Sablon marina in Saint-Malo, despite harbouring recreational vessels, were < 1 km from the main local ferry port.

Individuals were sampled from floating pontoons or buoys within the marinas for Jersey and British samples. Samples from Brittany were collected from under floating pontoons by divers. We attempted to leave a distance of at least one metre between each sampled individual to limit the chance of collecting related individuals in North English Channel populations, and random collection of samples over ca. 50m in South English Channel populations. However, this was not possible in all cases. We were only given permission to sample one pontoon at Brighton Marina due to adverse weather conditions at Isle of Wight individuals were only found on a small section of pontoon, and at NOCS individuals were only present on one floating pontoon.

**Appendix S3. DNA extraction**

We removed a piece of the branchial basket *in situ* in Jersey and in sites visited in Brittany, or *ex situ* in the laboratory for the remaining sites. When the branchial basket was removed *ex situ*, we attempted to minimise transport time between the field and the laboratory, ensuring this time did not exceed five hours. The collected samples were preserved in RNA*later*® Solution (Ambion) right after collection and stored in a -20°C freezer. DNA was eluted in 50 μl preheated (70ºC) Buffer BE and incubated for three minutes and centrifuged. This elution step was then repeated for high yield and concentration of nucleic acids.

**Appendix S4. Details on microsatellite genotyping methodology and rationale**

The highly polymorphic nature of microsatellites make them useful genetic markers, enabling assessment of genetic structure among populations and gene flow between populations [26]. Microsatellites have been developed from *C. robusta* and utilised in previous studies on *Ciona* *intestinalis* and *Ciona robusta* [27-29]. However, obtaining reliable data using these microsatellites has since proven difficult because of the large evolutionary divergence between the two species (ca. 14%; [30]) leading to null alleles when using primers designed on *C. robusta* to amplify DNA from *C. intestinalis*. For this reason, we amplified by PCR nine microsatellite loci specifically isolated for *C. intestinalis* [CiB32, CiB4, CiB45, CiB25, CiB47, CiB64, CiB13, CiB12 (Viard & Dubois, unpublished data; primers available upon request to F. Viard), and Cin12 [28] which has reliably amplified the two species when tested in experimental crosses (Viard & Dubois, unpublished data] (Table S1).

The PCR conditions used were based on a total reaction volume of 15 μL. Each reaction comprised of 3 μL 5 x buffer (Promega), 1.5 μL dNTP (2.5 mM), 1.2 μL MgCl2 (25 mM), 1.5 μL BSA (1 mg/ml), 0.1 μL Taq polymerase (Promega), 3.075 - 5.100 μL H2O, 2 μL template DNA, but differing primer concentrations per locus (Table S2). CiB47, CiB25, CiB12, and CiB64 were amplified together in a multiplex known as Multiplex 1 (M1, Table S2). CiB4, CiB32, CiB45, and Cin12 were amplified together in a multiplex known as Multiplex 2 (M2, Table S2). CiB13 was amplified separately (M3, Table S2).

There was an initial denaturation step in all PCR reactions at 95ºC for 5 min, followed by 10 cycles consisting of a denaturation step at 95ºC for 50 sec, an annealing step at 60ºC (- 1ºC per cycle) for 40 sec, and an elongation step at 72ºC for 40 sec. This was followed by 32 cycles, 28 cycles, or 25 cycles for Multiplex 1, Multiplex 2, and Multiplex 3 respectively, consisting of a denaturation step at 95ºC for 50 sec, an annealing step at 50ºC for 40 sec, and an elongation step at 72ºC for 40 sec. There was a final extension at 70ºC for 10 min. We estimated allele size using a capillary sequencer 3130xl Genetic Analyzer (Applied Biosystems) and the software Genemapper® v 4.0 (Applied Biosystems).

**Appendix S5. Details of data analysis methodology**

We calculated the number of amplified individuals (N), number of alleles (NA), and allelic richness per locus and population (AR) using FSTAT v. 2.9.3.2 [31]. We used Genepop On The Web v. 4.2 [32] to estimate observed (HO) and expected (HE) heterozygosities, and to test for linkage disequilibrium between pairs of loci in each population. We measured the number of private alleles using GenAlEx v.6.5 [33, 34]. We examined deviations from Hardy-Weinberg equilibrium (HWE) using the Genepop On The Web v. 4.2 [32] to compute the fixation index (FIS) with a test based on a permutation procedure using 10 000 bootstrap replicates.

We assessed pairwise population genetic differentiation using FST values [35] and their *P* values by running 10,000 permutations with Arlequin v.3.5.1.3 [36]. We also assessed the partition of genetic variance across populations using Jost’s *D* [37]. FST has previously been used to measure genetic differentiation between populations, however over the past 10 years this measure has come under criticism [37, 38]. In the case of loci with two alleles, *D* and FST give the same estimate [39]. But when using populations with more than two alleles, FST has been criticised because the relative role of mutation and migration becomes a key issue [37, 38]. This is particularly problematic when using microsatellite markers due to their high mutation rate and polymorphic nature that can be much higher than migration rate [40]. As a result, Jost [37] introduced another method of differentiation, *D*. Despite this, *D* depends heavily on the ratio between migration and mutation rate. Some authors have pointed out that Jost’s *D* may overestimate genetic differentiation and believe it not to be a valid replacement for FST [41]. For these reasons, both indices are included in the study. We used the package DEMEtics[42] in R (R Development Core Team, 2013) to calculate *D* values and their *P* values. If all the populations are in HWE, DEMEticsrandomises the alleles of a single locus within populations. If the populations are not in HWE, DEMEtics randomises the genotypes within populations (as alleles are not inherited independently) [43]. We obtained corrected *P* values of FST and *D* values using the Benjamini-Yuketieli method for multiple comparisons [44].

The Mantel Tests performed were based on geographical distances between sites using the ‘measure line’ tool in Google Earth (version 3.0, Google Inc., Menlo Park, CA, USA) with 10,000 permutations using Genepop On The Web v. 4.2 [32] as in Rousset [45].

We conducted a hierarchical analysis of molecular variance (AMOVA) using Arlequin [36] with 10 000 random permutations. The samples sites were grouped to Northern localities (Brighton, Gosport, Isle of Wight, Poole, Portsmouth, Town Quay, NOCS) and Southern localities (Jersey, Camaret, Brest, Aber-Wrac’h, Roscoff, Perros-Guirec, Saint-Quay-Portrieux, Saint-Malo). In order to visualise genetic clustering without a priori populations, we usedSTRUCTURE v. 2.3.4 [46], which employs a Bayesian, Markov Chain Monte Carlo (MCMC) algorithm. This algorithm infers the number of clusters (K) that maximises the genetic variation among clusters and minimises genetic variation within clusters, given the overall genetic variance within the dataset. We ran the software with the admixture setting, allowing for mixed ancestry. We applied the ‘loc prior’ model because it allows structure to be detected at low levels of population divergence and it is not biased towards detecting structure when there is none present [47]. To calculate the correct K value, we used an ad hoc quantity (ΔK) based on the second order rate of change of the likelihood function (ln Pr(X|K)) with respect to K [48]. We performed 20 independent replicates on K values ranging from 1 - 14 (the number of sampling sites) using a burn-in period (the length to run the simulation before collecting data to minimise the effect of the starting configuration) of 50,000 and 500,000 MCMC reps (the length to run the simulation after burn-in to get accurate parameter results) as in [49]. This was repeated when partitioning the dataset into Northern populations and Southern populations, however the 20 independent replicates used K values ranging from 1 to 7 and 1 to 8 respectively.

We performed a discriminant analysis of principal components (DAPC), which allows a visualisation of the between-population genetic variation [50]. We used the package *adegenet* v.1.4-2 [51] in R to perform the DAPC analysis. Principle component analysis (PCA) is able to identify genetic structures in large datasets without assuming an underlying population genetic model [50]. PCA however summarises the total variance among individuals (therefore including both variance between groups and within groups), and is unable to discriminate between groups. Discriminant Analysis (DA) is able to produce a model which partitions variation into a between-group and within-group component, maximising the former and minimising the latter [50]. DA, however, suffers limitations in that the number of variables (alleles) must be less than the number of observations (individuals) [50]. This is often not the case with highly polymorphic markers, and DA is hindered by correlations between variables [50]. DAPC overcomes these problems by transforming the data using PCA before using these PCA factors as variables for a discriminant analysis (DA). This ensures that the variables used in the DA are uncorrelated and does not lead to a loss of genetic information [50].

**Appendix S6. Genetic identification of species**

*Ciona robusta* and *C. intestinalis* have been recently accepted names, corresponding to two species previously merged within the nominal species “*Ciona intestinalis*”. Studies have shown morphological and genetic evidence [9, 52]. Diagnostic morphological characteristics are however not always easy to observe during sampling in the field. Thus, for each specimen collected, we confirmed the species identification by using a diagnostic mitochondrial molecular marker as detailed in Nydam & Harrison [53] and Bouchemousse *et al.* [17]. Screening via gel electrophoresis (Fig. S1) of the digested mtCOI gene by *Rsa*I confirmed that all samples analysed were *C. intestinalis*.

**Appendix S7. Detailed results of the genetic diversity analyses**

The generated data are freely available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.27fb8.

The number of alleles per locus ranged from seven at CiB25 to 24 at CiB4 and CiB47. Evidence for linkage disequilibrium was found only in the Portsmouth population between loci CiB32 and CiB4. As the two loci are not in disequilibrium in every population, and therefore the two loci are not in physical disequilibrium, they were both considered valid for the purpose of this study.

The mean expected heterozygosities (ranging from 0.658 to 0.727) were higher than the mean observed heterozygosities (ranging from 0.423 to 0.598) in all populations (Table S4). Global FIS values were significant for all populations, suggesting heterozygote deficiency (Table S4). CiB4 and CiB45 exhibited the highest deviation from Hardy-Weinberg equilibrium (HWE), with significant positive FIS values in 14 of the 15 populations (Table S4). The number of private alleles (i.e. alleles exclusively found in one site) varied between populations: Jersey contained 14 private alleles; NOCS contained four private alleles; Brighton, Brest, and Aber Wrac’h contained three private alleles each; Poole, Gosport, and Camaret contained two private alleles each; Isle of Wight, Perros-Guirec, Saint-Quay-Portrieux, and Saint-Malo contained one private allele each; Portsmouth, Town Quay, and Roscoff contained zero private alleles (Table S4).

Our results showed high levels of polymorphisms (Table S4). The expected heterozygosities calculated (range 0.658 – 0.727, average 0.690) were similar to those from other studies on *Ciona intestinalis* [HE = 0.775 – 0.871, average 0.819 [28]; HE = 0.510 – 0.875, average 0.760 [29]]. As Zhan et al. [28] identified, these findings were consistent with previous studies on individuals of the genus *Ciona*, which also described high levels of genetic diversity [30, 54, 55]. Indeed, *C. intestinalis’* congener, *Ciona savignyi*, has been described as exhibiting ‘the highest structural polymorphism ever comprehensively quantified in a multicellular organism’ [56, 57]. The expected heterozygosities detected in this study were higher than those in other similar taxa including *Styela clava* [0.449 < HE < 0.626 [58-60]], *Pyura chilensis* [0.219 < HE < 0.298 [61]], and *Corella eumyota* [0.13 [62]].

All of the studied sites displayed significant heterozygote deficiencies (FIS = 0.140 – 0.384). Heterozygote deficiencies may be indicative of null alleles [alleles at a microsatellite locus that fail to amplify to detectable levels by PCR [63]] or non-random mating between individuals due to biological factors including inbreeding and Wahlund effect [64]. However, discerning among these possible factors is extremely challenging (see below). Sources of null alleles include poor primer annealing due to mutations in flanking regions [63], differential amplification of size-variant alleles (whereby smaller alleles are amplified more readily than larger alleles) [65], and low or inconsistent DNA template quality [63]. Null alleles can seemingly reduce genetic diversity within populations and therefore lead to erroneous results of heterozygote deficiency. They can also overestimate FST under some circumstances, for instance when gene flow is limited [66]. The loci that displayed the highest numbers of non-amplifying individuals (17 and 22 individuals for CiB4 and CiB45 respectively) were the two loci that displayed heterozygous deficiencies in all populations. Despite this, PCR amplification was successful (> 95% across populations), with a greater success than what has been reported by Zhan et al. [28]. Another possibility is the incorrect scoring of allele bins, which is also unlikely here as the same person scored each individual using pre-determined bin sets. Regarding the possibility of heterozygote deficiencies as a result of non-random mating between individuals, inbreeding is theoretically plausible for *C. intestinalis* due to its limited dispersal capabilities [22, 25]. Studies on the larval dispersion of *C. intestinalis* in Sweden and Denmark have shown that egg and larvae numbers in the water column during spawning may be scarce due to epibenthic retention of eggs in mucus strings (egg-strings) [22, 25]. Additionally, laboratory experiments on egg-strings in Petri dishes suggest only 40 - 60% of larvae hatching from these egg-strings escape to the plankton, while the rest metamorphose in the nearby mucus [22]. It has also been shown that *C. intestinalis* larvae have a tendency to settle close to or even on top of adults, forming multigenerational clusters [67]. Additionally, all individuals in this study were collected from enclosed or semi-enclosed marinas where self-recruitment is dominant and inbreeding may be more common than in open habitats [28, 59]. Bouchemousse et al. [17] showed less than 5% of fertilization success in self-crosses. Inbreeding should lead to heterozygote deficiency at all loci, and none of the sampled populations displayed this pattern (Table S4), therefore it is unlikely to have caused the observed heterozygote deficiencies [59, 68]. For these reasons, the significant high values of FIS are unlikely to be explained by self-fertilisation. The Wahlund effect describes the reduction of heterozygosity caused by cryptic subpopulations. If two or more subpopulations have differing allele frequencies, overall observed heterozygosity will decrease as compared to expected values, even if individual subpopulations are in Hardy-Weinberg equilibrium. Analyses for both spatial and temporal Wahlund effects were beyond the scope of this study. Studies to evaluate the Wahlund effect would require specific sampling strategies. To determine the effect of a spatial Wahlund effect, we would be required to perform additional studies on genotype distribution at each site, with the precise location of each sampled individual being noted [69]. To determine the effect of a temporal Wahlund effect, a monthly sampling of individuals at these two sites would be required, followed by a study of temporal trends and patterns of genetic differentiation. The observed heterozygote deficiencies, rather than being a result of one of these factors, may therefore be a result of additive effects of a number of these factors [70].

**Appendix S8. AMOVA results**

A hierarchical AMOVA was carried out to compare the genetic variance between sub-regional groups of populations. It showed that most genetic variation was found within populations. Variations among groups were non-significant and the variation among populations within groups was small but significant (Table S5).

**Appendix S9. Genetic clustering without *a priori* populations**

Regarding the analyses conducted with STRUCTURE, the ad hoc value ΔK suggested a two-cluster model (K = 2) as the most parsimonious outcome (Fig. 2A). The averaged proportional membership of individuals sampled in Jersey to one cluster was 83% (Fig 2A, green), whilst Brighton, Gosport, Isle of Wight, Poole, Portsmouth, Town Quay, Camaret, Brest, Aber Wrac’h, Roscoff, Perros-Guirec, Saint-Quay-Portrieux, and Saint-Malo included the majority of individuals belonging to the other cluster with a probability of assignment between 99% and 84% (Fig. 2A, red). Individuals from NOCS also mostly assigned to one cluster, but the probability of assignment was much lower (65%) (Fig. 2A, green).

When analysing each region (Northern vs Southern excluding Jersey) separately, STRUCTURE suggested a three-cluster model (K = 3) as the most parsimonious possibility for the Northern region, with a similar proportion of assignment to each cluster in each population (Fig. S2A). STRUCTURE suggested a two-cluster model (K = 2) for the Southern region (Fig. S2B).

The individuals belonging to Brest and Aber Wrac’h had a higher percentage of individuals assigned to one cluster (green in Fig. S2B) while Roscoff, Perros-Guirec, and Saint-Malo had the majority of individuals belonging to the other cluster (red in Fig. S2B). Individuals from Camaret and Saint-Quay-Portrieux had mixed assignment between both clusters (Fig. S2B).

The scatterplot of the first two axes of the DAPC (the first two components of the DA) showed three clusters of populations. A cluster including Jersey and NOCS, a cluster including Gosport, Isle of Wight, Poole, Portsmouth, Town Quay, and Saint-Quay-Portrieux, and a third cluster including Brighton, Camaret, Brest, Aber Wrac’h, Roscoff, Perros-Guirec, and Saint-Malo (Fig. 2B). The primary axis (x-axis) separated Jersey from the rest of the locations. The secondary axis (y-axis) further separated Gosport, Isle of Wight, Poole, Portsmouth, Town Quay, and Saint-Quay-Portrieux from the Brighton, Camaret, Brest, Aber Wrac’h, Roscoff, Perros-Guirec, and Saint-Malo. 65.5% of individuals were correctly reassigned to their original group (Fig. 2B).

We reanalysed the data using six groups (Brighton; Saint-Quay-Portrieux; a group including Camaret, Brest, Aber Wrac’h, Roscoff, Perros-Guirec, and Saint-Malo [known as ‘FRA’]; Jersey; NOCS and a group including Gosport, Isle of Wight, Poole, Portsmouth, and Town Quay [known as ‘ENG’]) for DAPC analysis (Fig. S3).

The primary axis (x-axis) again separated Jersey and NOCS from the rest of the locations. The secondary axis (y-axis) further separated ‘ENG’ and Saint-Quay-Portrieux from Brighton and ‘FRA’. 76.0% of individuals were correctly reassigned to their original group (Fig S3).

Mantel tests comparing populations per regions found no correlation between genetic and geographic distance (northern sites: r = 0.057, *P* = 0.349, Fig. S4C; southern sites: r = 0.189, *P* = 0.123, Figs. S4D).

This study shows how genetic tools can help in disentangling dissimilar dispersal pathways across a highly reshuffled species ranges. Our study used population genetics approaches examining urban habitats (marinas and harbours) that are usually examined to study the colonisation of non-indigenous species. Therefore, whilst previous population genetic studies focus on non-indigenous species, this study provides a good understanding of the connectivity among populations established in the native range. Genetic tools have previously been used to elucidate formerly undetected genetic differentiation of species in native ranges [71, 72]. Our study additionally proposes that different types of vectors influence the outcome of translocations. Murray *et al.* [73] found recreational boating to be a key vector in the introduction of non-indigenous species in British Columbia, a result supported by this study. This vector comes in addition to other possible vectors like commercial vessels, which may transport organisms by means of ballast water. However, the latter are unlikely to be very important vectors for organisms with short-lived planktonic stages [74].

The presence of private alleles is generally attributed to the existence of isolation among populations and/or recent species expansions [69, 75, 76]. Whilst private alleles have been suggested to correlate with genetic structure [75], this has only been validated using simple models such as stepwise mutation model [77]. As such care should be taken when interpreting the presence of private alleles.

Low gene flow between conspecific populations can result in genetic heterogeneity between demes both spatially and temporally. This low gene flow, the consequence of limited migration between populations, can be due to physical barriers such as ocean currents or biological factors including spawning season and larval planktonic duration [78]. Accordingly, the limited natural dispersal capability of *C. intestinalis* [23-25] is expected to result in genetic differentiation among the populations sampled in this study. It therefore comes as a surprise that many pairwise comparisons of genetic differentiation among populations showed no significant differentiation [44 out of 105 (41.9%) *D* values; 51 out of 105 (48.6%) FST values (Table 2)]. Weak pattern of genetic differentiation can be produced with limited gene flow when species display large effective population size [79], which is likely in the study species, characterized by high fertility and external fertilization. Alternatively, this indicates that enough larvae travel between ‘ENG’ populations so as to prevent the drifting apart of allele frequencies. As there is a general current flow eastwards in the English Channel [80], it may be assumed that natural dispersal between populations on the south coast of England would be from west (Poole) to east (Brighton). However, studies have shown gene flow between populations is not always supported as that hypothesised by dispersal via the dominant marine currents [for example in North America [81]]. All populations sampled in this study were within enclosed or semi-enclosed waters (marinas). Water currents and gyres associated with embayments or banks tend to limit dispersal of larvae from these habitats by acting as a genetic barrier [82, 83]. In Prince Edward Island, Canada, *C. intestinalis* is a highly invasive species that is incapable of natural dispersal between bays typically separated by 10s km [23]. Nevertheless, *C. intestinalis* has been documented in bays separated by this distance, which suggests that dispersal is accomplished by anthropogenic activities such as aquaculture activity or recreational boating [23]. Therefore, a probable reason for the low genetic differentiation among ‘ENG’ populations (recreational marinas separated by 10s km) is anthropogenic dispersal via recreational boating. The effect of recreational boating has been shown to be a major driver of genetic structure in ascidians in marinas [84] partly due to the unregulated nature of recreational boating hull fouling [73]. It should be reiterated that we studied a species established in urban areas (i.e. marinas and ports), not in natural habitats. This alters the likelihood that individuals would be transported by human-mediated activities, and suggests that the major currents at the Channel level are unlikely to play a significant role. Thus the results of this study are highly specific, and unlikely to be true for many other species established in natural habitats. It can be argued that the observed genetic pattern may not be an alteration of a pre-existing, natural genetic landscape, but rather be an independent structure of new populations superimposed on the natural genetic landscape, and not derived directly from it.

Jersey was genetically isolated from each population along the south coast of England and North coast of France other than NOCS. This was observed by pairwise comparisons (*D* values and FST values, Table 2), as well as STRUCTURE and DAPC (Fig. 2A/B). This genetic differentiation is unexpected as commercial anthropogenic transport links Jersey to Saint-Malo [85] and Jersey to Poole [86]. This apparent low gene flow could be explained by *C. intestinalis* not being transported by ferry, as the populations studied are established in nearby marinas but not in ferry ports. Additionally, the marinas at Saint-Malo and Poole may have been founded by a different set of colonisers than Jersey. *Ciona intestinalis* has been known to invade new regions rapidly; the time between identification and establishment as the dominant fouling organism in an estuary has been documented to be as low as two years [20]. When *C. intestinalis* dominates rapidly, it is thought to be due to a recruitment advantage whereby reproduction starts at a lower temperature than other species [20]. In this study, if *C. intestinalis* is being introduced between Jersey and Poole, and Jersey and Saint-Malo, it may not demonstrate such an obvious recruitment advantage over conspecifics, and therefore it may not colonise new sites as readily. To support this, there is also a ferry service that operates between Jersey and Portsmouth via Guernsey (an island *c.* 50 km from Jersey), and neither Portsmouth nor Gosport (the two sites closest to Portsmouth ferry port) show evidence of genetic relatedness with Jersey. This therefore suggests that ferries may have a limited effect on the genetic shuffling of *C. intestinalis*. There is high intensity of yacht traffic between Jersey and Saint-Malo (Appendix S4), however this less important compared to shipping traffic between Southern England and Northwest France. Of further interest is the apparent genetic homogeneity between Jersey and NOCS (Table 2). There is no direct commercial link (i.e. ferry service) between these two sites, like there is between Jersey and Poole, to explain the observed pattern [87]. Additionally, NOCS is only *c.* 2.5 km away from Town Quay, a site that shows significant genetic differentiation from both Jersey and NOCS (Table 2). Southampton, the city where NOCS and Town Quay are situated, is a major harbour where ferries and research vessels can be found. A reason for this observed pattern may be differences in shipping activity. These different vectors may travel to and from different locations. Moreover, whilst the sampling location in Jersey was a marina harbouring recreational vessels, this marina was < 1 km from the main ferry port to the island. Recreational shipping is less likely to visit distant waters than larger commercial and research vessels. The locations visited by large ferries from Jersey and vessels into NOCS may be similar or overlap and lead to the apparent lack of genetic differentiation between these two sites. There is a cargo vessel that travels between Jersey and Southampton three times a week. Unfortunately, as the data provided on yachts visiting Jersey is not accurate enough to differentiate between the two sites in Southampton, and therefore cannot specifically explain a direct link between Jersey and NOCS, however it may suggest indirect links. For example, the yachts visiting Jersey that have travelled from the Isle of Wight (Appendix S5) may not have been from the same marina as that sampled in this study, but may provide a possibility for a stepping stone dispersal system between Jersey and NOCS.

The DAPC suggests some individuals from other sites were genetically similar to individuals from Jersey, as seen by individuals inside Jersey’s ellipse (Fig. 2B). These individuals came from Saint-Malo, Poole, Town Quay, Roscoff (2 individuals), Gosport, Brighton, and Brest. A few yachts connecting these sites could explain this observed pattern.

The DAPC (Fig S3) observed Brighton to be differentiated from other ‘ENG’ sites, however this is not consistent with pairwise FST or *D* comparisons (Table 2), and STRUCTURE did not identify Brighton as a separate cluster (Fig. 2A). Whether the DAPC result is enough to distinguish Brighton as a separate ‘population’ from ‘ENG’ sites is uncertain. A similar result was observed between Saint-Quay-Portrieux and ‘FRA’ sites (DAPC, Fig S3); whilst this observed differentiation was not supported by STRUCTURE (Fig. 2A), it was supported by pairwise FST and *D* comparisons (Table 2). Many different definitions of a ‘population’, from both an evolutionary and ecological perspective, are found in the literature (reviewed in Waples & Gaggiotti [88]). As population differentiation occurs along a continuum, it is often difficult to precisely determine a cut off point of when subunits are differentiated enough to be considered ‘populations’ [88]. The configuration of sample sites can limit gene flow between populations, even in the presence human-mediated transport [29, 59]. Brighton was the most enclosed marina sampled along the south coast of England in this study, which strengthens the argument that the pattern observed in the DAPC plot is due to anthropogenic transport and not natural.

**Appendix S10. Marina acknowledgements**

We thank Sparkes Marina Development Limited, Premier Marina Brighton, Shepards Wharf Marina, Gosport Marina, Parkstone Yacht Club, Town Quay, and Jersey Marinas for allowing sampling. We also thank the Brittany marina operators for allowing us to conduct the surveys and sampling.

**Table S1.** List of loci used with locus name; repeat array in original sequence; allele size range (from populations in this study); and the source of the primer.

|  |  |  |  |
| --- | --- | --- | --- |
| Locus | Repeat array | Allele size range (No base pairs) | Primer source |
|
| CiB4 | (TGT)12 | 127 - 231 | Viard & Dubois (Unpublished) |
|
| CiB12 | (CA)8 | 172 - 194 | Viard & Dubois (Unpublished) |
|
| CiB13 | (GA)8 | 160 - 171 | Viard & Dubois (Unpublished) |
|
| CiB25 | (GTGGTT)8 | 173 - 204 | Viard & Dubois (Unpublished) |
|
| CiB32 | (ACA)3 | 156 - 190 | Viard & Dubois (Unpublished) |
|
| CiB45 | (TTG)6 | 93 - 117 | Viard & Dubois (Unpublished) |
|
| CiB47 | (TGT)6 | 88 - 171 | Viard & Dubois (Unpublished) |
|
| CiB64 | (CGT)6 | 239 - 251 | Viard & Dubois (Unpublished) |
|
| Cin12 | (CTT)20 | 168 - 259 | [28] |
|

**Table S2.** PCR conditions for each multiplex. ‘F\*\*’ represents forward fluorescent primer; ‘F’ represents forward non-fluorescent primer; ‘R’ represents reverse primer.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Locus | Primers | Volume (μl) |
| M1 | CiB47 | F\*\* 10 μM | 0.200 |
| F 10 μM | 0.100 |
| R 10 μM | 0.300 |
| CiB25 | F\*\* 10 μM | 0.200 |
| F 10 μM | 0.100 |
| R 10 μM | 0.300 |
| CiB12 | F\*\* 2 μM | 0.100 |
| F 10 μM | 0.280 |
| R 10 μM | 0.300 |
| CiB64 | F\*\* 10 μM | 0.100 |
| F 10 μM | 0.200 |
| R 10 μM | 0.300 |
| M2 | CiB4 | F\*\* 5 μM | 0.200 |
| F 10 μM | 0.200 |
| R 10 μM | 0.300 |
| CiB32 | F\*\* 5 μM | 0.050 |
| F 10 μM | 0.275 |
| R 10 μM | 0.300 |
| CiB45 | F\*\* 5 μM | 0.100 |
| F 10 μM | 0.250 |
| R 10 μM | 0.300 |
| Cin12 | F\*\* 5 μM | 0.100 |
| F 10 μM | 0.250 |
| R 10 μM | 0.300 |
| M3 | CiB 13 | F\*\* 10 μM | 0.200 |
| F 10 μM | 0.100 |
| R 10 μM | 0.300 |

**Table S3.** The number of yachts that visited Jersey between 2011 - 2015 and the location of their last port.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Year** | | | | |
| **Location** | **2011** | **2012** | **2013** | **2014** | **2015** |
| Brighton | 1 | 2 | 1 | 1 | 2 |
| Gosport | 3 | 4 | 5 | 6 | 1 |
| Isle of Wight | 5 | 3 | 5 | 2 | 5 |
| Poole | 10 | 24 | 5 | 4 | 5 |
| Portsmouth | 6 | 4 | 16 | 5 | 3 |
| Southampton | 8 | 3 | 8 | 17 | 6 |
| Camaret | 3 | 6 | 2 | 4 | 3 |
| Brest | 11 | 4 | 4 | 8 | 5 |
| Aber Wrac'h | 3 | 0 | 4 | 2 | 1 |
| Roscoff | 1 | 14 | 16 | 24 | 15 |
| Perros-Guirec | 23 | 15 | 27 | 16 | 8 |
| St Quay-Portrieux | 210 | 183 | 181 | 183 | 140 |
| St Malo | 1174 | 1053 | 985 | 1071 | 923 |

**Table S4.** Genetic variation in the sampled sites. Number of amplified individuals (N); number of alleles (NA); private alleles (if any) are indicated inside parentheses; allelic richness per locus and population (AR) based on a minimum amplified sample size (over all loci) of 14 diploid individuals; observed (HO) and expected (HE) heterozygosities; and fixation index (FIS). Significant FIS values are in bold. Means over loci (or global value for FIS) are also indicated.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Locus |  |  |  |  |  |  |  |  |  |
|  | CiB32 | CiB4 | CiB45 | Cin12 | CiB25 | CiB47 | CiB64 | CiB13 | CiB12 | Mean |
| Brighton (BTN) |  |  |  |  |  |  |  |  |  |  |
| N | 33 | 33 | 32 | 33 | 33 | 33 | 33 | 33 | 33 | 32.889 |
| NA | 10 | 12 (1) | 10 (1) | 15 (1) | 5 | 17 | 3 | 3 | 8 | 9.222 |
| AR | 7.289 | 7.786 | 8.124 | 10.288 | 4.160 | 12.043 | 2.813 | 2.423 | 7.257 | 6.909 |
| Ho | 0.758 | 0.606 | 0.406 | 0.848 | 0.182 | 0.788 | 0.242 | 0.303 | 0.788 | 0.547 |
| He | 0.821 | 0.770 | 0.830 | 0.839 | 0.533 | 0.909 | 0.335 | 0.307 | 0.840 | 0.687 |
| Fis | 0.078 | **0.216** | **0.515** | -0.012 | **0.662** | 0.135 | 0.280 | 0.014 | 0.063 | **0.207** |
| Gosport (GOS) |  |  |  |  |  |  |  |  |  |  |
| N | 33 | 33 | 30 | 33 | 33 | 33 | 33 | 33 | 33 | 32.667 |
| NA | 11 | 12 | 9 | 12 | 4 | 19 | 4 (1) | 5 (1) | 12 | 9.778 |
| AR | 8.873 | 8.961 | 8.032 | 9.830 | 3.891 | 13.317 | 3.328 | 3.327 | 8.588 | 7.572 |
| Ho | 0.939 | 0.576 | 0.400 | 0.818 | 0.273 | 0.848 | 0.091 | 0.455 | 0.758 | 0.573 |
| He | 0.861 | 0.833 | 0.869 | 0.843 | 0.538 | 0.912 | 0.297 | 0.518 | 0.841 | 0.724 |
| Fis | -0.093 | **0.312** | **0.544** | 0.030 | **0.497** | 0.071 | **0.697** | 0.125 | 0.101 | **0.211** |
| Isle of Wight (IOW) |  |  |  |  |  |  |  |  |  |  |
| N | 24 | 24 | 24 | 23 | 24 | 24 | 24 | 24 | 24 | 23.889 |
| NA | 9 | 9 | 9 | 12 | 3 | 14 | 3 | 3 | 13 (1) | 8.333 |
| AR | 7.958 | 7.487 | 8.232 | 10.154 | 2.934 | 11.799 | 2.583 | 2.934 | 9.630 | 7.079 |
| Ho | 0.833 | 0.500 | 0.417 | 0.783 | 0.292 | 0.917 | 0.292 | 0.417 | 0.833 | 0.587 |
| He | 0.849 | 0.748 | 0.833 | 0.832 | 0.504 | 0.915 | 0.393 | 0.504 | 0.843 | 0.714 |
| Fis | 0.019 | **0.337** | **0.505** | 0.061 | **0.427** | -0.002 | 0.261 | 0.177 | 0.012 | **0.181** |

**Table S4** continued

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Locus |  |  |  |  |  |  |  |  |  |
|  | CiB32 | CiB4 | CiB45 | Cin12 | CiB25 | CiB47 | CiB64 | CiB13 | CiB12 | Mean |
| Poole (POO) |  |  |  |  |  |  |  |  |  |  |
| N | 33 | 32 | 31 | 33 | 32 | 32 | 32 | 33 | 33 | 32.333 |
| NA | 9 | 11 | 9 | 16 | 4 | 15 | 6 (2) | 4 | 11 | 9.444 |
| AR | 7.874 | 8.652 | 7.640 | 10.587 | 3.656 | 10.414 | 4.506 | 2.848 | 8.233 | 7.157 |
| Ho | 0.879 | 0.469 | 0.355 | 0.848 | 0.281 | 0.781 | 0.313 | 0.576 | 0.879 | 0.598 |
| He | 0.838 | 0.821 | 0.839 | 0.861 | 0.434 | 0.871 | 0.444 | 0.508 | 0.851 | 0.718 |
| Fis | -0.050 | **0.433** | **0.581** | 0.014 | **0.355** | 0.105 | **0.300** | -0.136 | -0.033 | **0.170** |
| Portsmouth (POR) |  |  |  |  |  |  |  |  |  |  |
| N | 33 | 33 | 32 | 32 | 33 | 33 | 32 | 33 | 33 | 32.667 |
| NA | 11 | 10 | 7 | 12 | 4 | 14 | 4 | 3 | 10 | 8.333 |
| AR | 8.017 | 7.176 | 6.358 | 9.828 | 3.913 | 11.475 | 3.173 | 2.672 | 8.345 | 6.773 |
| Ho | 0.788 | 0.545 | 0.281 | 0.875 | 0.273 | 0.788 | 0.188 | 0.394 | 0.848 | 0.553 |
| He | 0.729 | 0.795 | 0.825 | 0.878 | 0.537 | 0.912 | 0.232 | 0.446 | 0.848 | 0.689 |
| Fis | -0.083 | **0.318** | **0.663** | 0.004 | **0.496** | 0.138 | 0.193 | 0.118 | -0.001 | **0.200** |
| Town Quay (TNQ) |  |  |  |  |  |  |  |  |  |  |
| N | 22 | 22 | 21 | 22 | 22 | 22 | 22 | 21 | 21 | 21.667 |
| NA | 8 | 8 | 8 | 10 | 4 | 11 | 2 | 4 | 9 | 7.111 |
| AR | 7.592 | 7.131 | 7.450 | 9.040 | 3.860 | 9.878 | 2.000 | 3.333 | 8.195 | 6.678 |
| Ho | 0.818 | 0.409 | 0.333 | 0.773 | 0.318 | 0.864 | 0.227 | 0.667 | 0.810 | 0.580 |
| He | 0.856 | 0.820 | 0.846 | 0.860 | 0.503 | 0.884 | 0.384 | 0.517 | 0.870 | 0.727 |
| Fis | 0.046 | **0.507** | **0.612** | 0.104 | **0.373** | 0.023 | 0.413 | -0.299 | 0.071 | **0.206** |

**Table S4** continued

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Locus |  |  |  |  |  |  |  |  |  |
|  | CiB32 | CiB4 | CiB45 | Cin12 | CiB25 | CiB47 | CiB64 | CiB13 | CiB12 | Mean |
| National Oceanography Center (NOCS) | | | | |  |  |  |  |  |  |
| N | 18 | 14 | 15 | 16 | 18 | 17 | 18 | 17 | 16 | 16.556 |
| NA | 10 | 9 | 8 | 10 (2) | 3 | 13 | 2 | 3 (2) | 7 | 8.444 |
| AR | 9.007 | 9.000 | 7.989 | 9.351 | 2.956 | 12.031 | 1.778 | 2.973 | 6.976 | 6.896 |
| Ho | 0.722 | 0.500 | 0.400 | 0.688 | 0.333 | 0.941 | 0.059 | 0.471 | 0.750 | 0.540 |
| He | 0.811 | 0.831 | 0.844 | 0.796 | 0.408 | 0.911 | 0.059 | 0.483 | 0.847 | 0.665 |
| Fis | 0.112 | **0.407** | **0.535** | 0.141 | 0.187 | -0.034 | 0.000 | 0.027 | 0.118 | **0.193** |
| Jersey (JER) |  |  |  |  |  |  |  |  |  |  |
| N | 68 | 63 | 62 | 68 | 69 | 68 | 69 | 69 | 68 | 67.111 |
| NA | 11 | 15 | 12 (5) | 14 (6) | 6 | 19 (1) | 4 | 3 (1) | 13 (1) | 10.778 |
| AR | 8.288 | 8.606 | 8.689 | 9.448 | 4.568 | 11.070 | 2.801 | 2.203 | 8.557 | 7.137 |
| Ho | 0.750 | 0.619 | 0.516 | 0.794 | 0.333 | 0.853 | 0.203 | 0.406 | 0.765 | 0.582 |
| He | 0.831 | 0.828 | 0.844 | 0.814 | 0.492 | 0.890 | 0.348 | 0.462 | 0.843 | 0.706 |
| Fis | 0.099 | **0.237** | **0.390** | 0.025 | **0.324** | 0.042 | **0.418** | 0.122 | 0.094 | **0.174** |
| Camaret (CAM) |  |  |  |  |  |  |  |  |  |  |
| N | 33 | 33 | 32 | 33 | 33 | 32 | 30 | 33 | 33 | 32.444 |
| NA | 11 | 10 (2) | 8 | 13 | 5 | 13 | 3 | 5 | 10 | 8.667 |
| AR | 8.238 | 7.607 | 7.525 | 9.050 | 3.664 | 10.496 | 2.458 | 3.802 | 8.347 | 6.799 |
| Ho | 0.727 | 0.576 | 0.500 | 0.727 | 0.424 | 0.781 | 0.200 | 0.333 | 0.826 | 0.566 |
| He | 0.827 | 0.779 | 0.832 | 0.730 | 0.537 | 0.901 | 0.239 | 0.389 | 0.758 | 0.666 |
| Fis | 0.122 | **0.264** | **0.403** | 0.003 | 0.213 | **0.135** | 0.165 | 0.145 | 0.084 | **0.173** |

**Table S4** continued

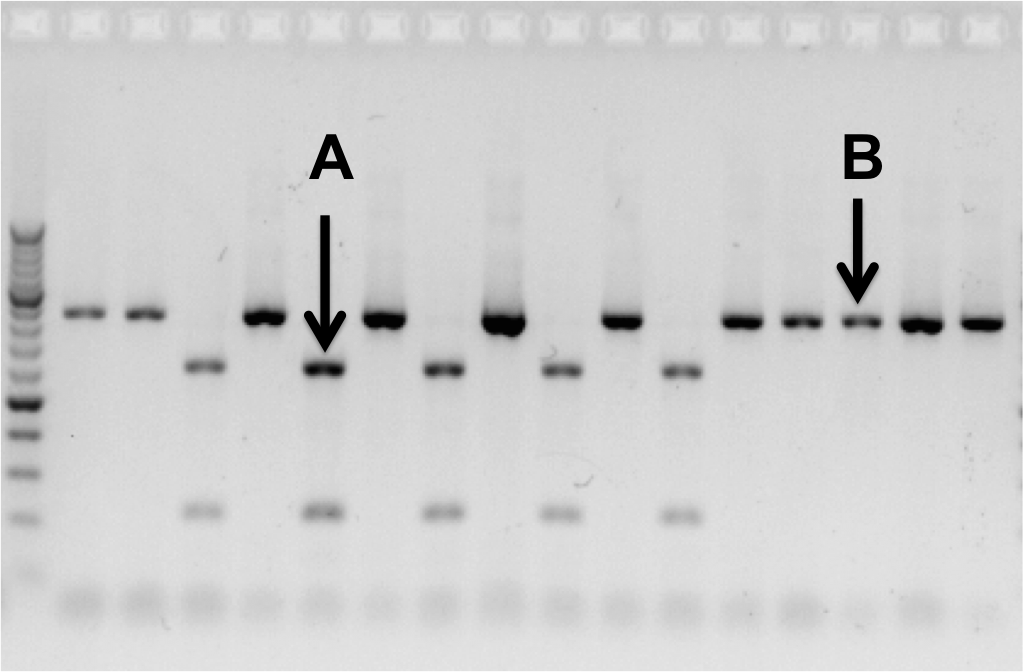
|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Locus |  |  |  |  |  |  |  |  |  |
|  | CiB32 | CiB4 | CiB45 | Cin12 | CiB25 | CiB47 | CiB64 | CiB13 | CiB12 | Mean |
| Brest (BC) |  |  |  |  |  |  |  |  |  |  |
| N | 33 | 33 | 32 | 33 | 33 | 33 | 33 | 33 | 33 | 32.889 |
| NA | 12 (2) | 11 (1) | 9 | 13 | 5 | 13 | 2 | 4 | 11 | 8.889 |
| AR | 8.940 | 7.870 | 7.280 | 9.475 | 4.283 | 10.568 | 2.000 | 2.818 | 9.676 | 6.990 |
| Ho | 0.727 | 0.546 | 0.438 | 0.667 | 0.303 | 0.879 | 0.394 | 0.182 | 0.939 | 0.564 |
| He | 0.858 | 0.816 | 0.758 | 0.780 | 0.473 | 0.892 | 0.388 | 0.222 | 0.876 | 0.674 |
| Fis | **0.154** | **0.335** | **0.427** | **0.147** | **0.363** | 0.015 | -0.015 | 0.185 | -0.073 | **0.165** |
| Aber Wrac'h (AW) |  |  |  |  |  |  |  |  |  |  |
| N | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 |
| NA | 12 | 7 | 10 (2) | 12 (1) | 5 | 14 | 3 | 4 | 10 | 8.556 |
| AR | 9.755 | 5.775 | 8.711 | 8.792 | 4.745 | 11.113 | 2.451 | 3.462 | 8.012 | 6.980 |
| Ho | 0.839 | 0.387 | 0.452 | 0.645 | 0.387 | 0.839 | 0.355 | 0.258 | 0.935 | 0.566 |
| He | 0.885 | 0.711 | 0.853 | 0.785 | 0.521 | 0.901 | 0.323 | 0.266 | 0.852 | 0.677 |
| Fis | 0.053 | **0.460** | **0.475** | 0.180 | **0.261** | 0.070 | -0.100 | 0.030 | -0.100 | **0.166** |
| Roscoff (RB) |  |  |  |  |  |  |  |  |  |  |
| N | 30 | 30 | 31 | 31 | 31 | 30 | 31 | 31 | 31 | 30.667 |
| NA | 8 | 7 | 10 | 13 | 5 | 10 | 3 | 5 | 13 | 8.222 |
| AR | 6.739 | 6.023 | 9.054 | 9.999 | 4.324 | 8.306 | 2.684 | 3.596 | 10.612 | 6.815 |
| Ho | 0.733 | 0.567 | 0.710 | 0.839 | 0.290 | 0.700 | 0.194 | 0.290 | 0.806 | 0.570 |
| He | 0.737 | 0.766 | 0.863 | 0.833 | 0.561 | 0.821 | 0.182 | 0.314 | 0.871 | 0.661 |
| Fis | 0.005 | 0.263 | 0.180 | -0.007 | **0.486** | 0.149 | -0.062 | 0.077 | 0.076 | **0.140** |

**Table S4** continued

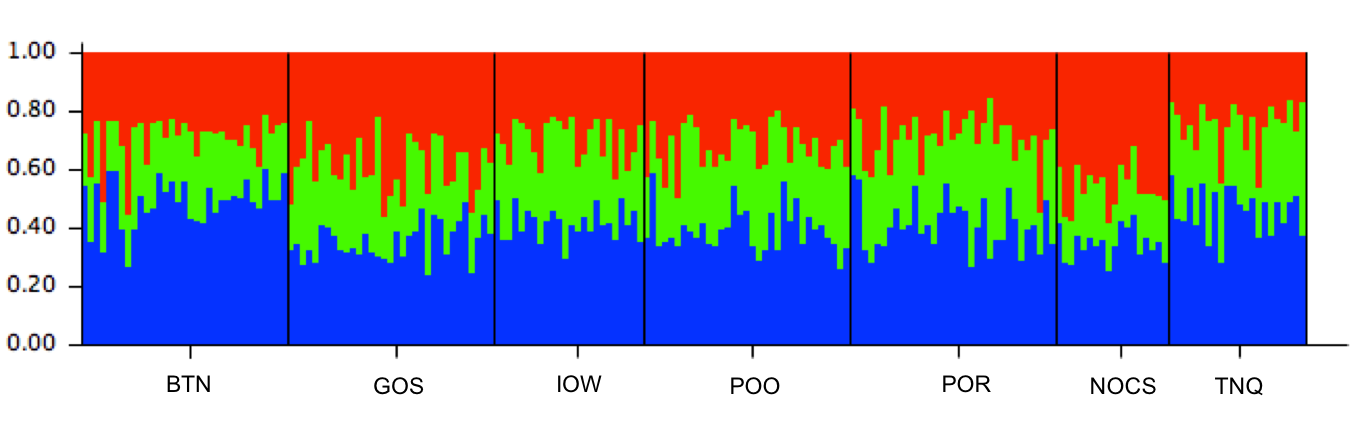
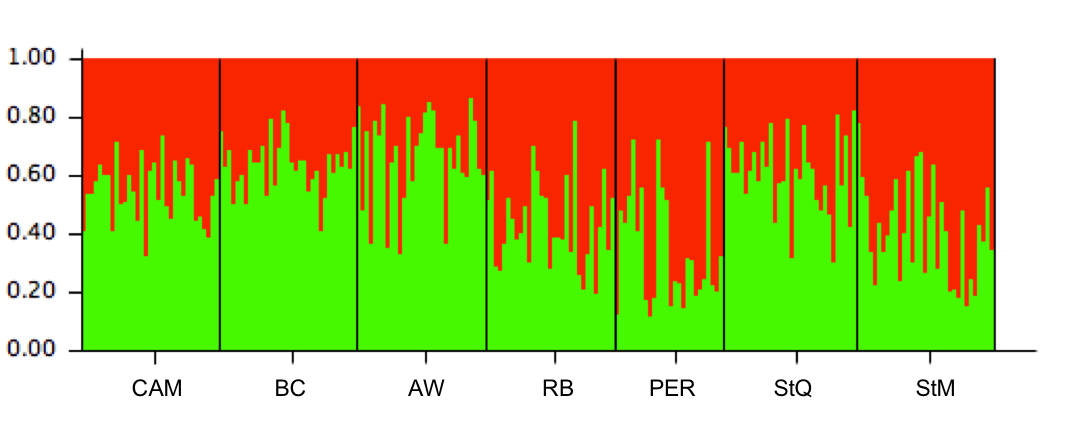
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Locus | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
|  | CiB32 | | CiB4 | | CiB45 | | Cin12 | | CiB25 | | CiB47 | | CiB64 | | CiB13 | | CiB12 | | Mean | |
| Perros-Guirec (PER) |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| N | 26 | | 24 | | 26 | | 26 | | 25 | | 23 | | 26 | | 26 | | 25 | | 25.222 | |
| NA | 5 | | 8 | | 8 | | 11 | | 6 (1) | | 12 | | 2 | | 3 | | 8 | | 7.000 | |
| AR | 4.976 | | 6.284 | | 7.575 | | 9.070 | | 5.170 | | 10.510 | | 2.000 | | 2.538 | | 6.914 | | 6.115 | |
| Ho | 0.462 | | 0.375 | | 0.346 | | 0.692 | | 0.240 | | 0.522 | | 0.308 | | 0.423 | | 0.440 | | 0.423 | |
| He | 0.765 | | 0.704 | | 0.835 | | 0.778 | | 0.581 | | 0.850 | | 0.362 | | 0.429 | | 0.825 | | 0.681 | |
| Fis | **0.402** | | **0.473** | | **0.590** | | 0.112 | | **0.592** | | **0.392** | | 0.153 | | 0.014 | | **0.472** | | **0.384** | |
| Saint-Quay-Portrieux (StQ) | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| N | 32 | | 30 | | 31 | | 32 | | 32 | | 31 | | 31 | | 32 | | 32 | | 31.444 | |
| NA | 8 (1) | | 9 | | 6 | | 13 | | 4 | | 15 | | 3 | | 3 | | 10 | | 7.889 | |
| AR | 6.455 | | 7.154 | | 5.396 | | 9.686 | | 3.436 | | 10.692 | | 2.703 | | 2.998 | | 8.068 | | 6.288 | |
| Ho | 0.656 | | 0.533 | | 0.516 | | 0.781 | | 0.219 | | 0.839 | | 0.226 | | 0.375 | | 0.625 | | 0.530 | |
| He | 0.782 | | 0.792 | | 0.768 | | 0.772 | | 0.565 | | 0.880 | | 0.450 | | 0.518 | | 0.821 | | 0.705 | |
| Fis | 0.163 | | **0.330** | | **0.332** | | -0.012 | | **0.617** | | 0.048 | | **0.502** | | 0.279 | | **0.242** | | **0.252** | |
| Saint-Malo (StM) |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| N | 33 | | 32 | | 32 | | 33 | | 32 | | 31 | | 31 | | 33 | | 33 | | 32.222 | |
| NA | 10 | | 11 (1) | | 9 | | 11 | | 5 | | 13 | | 2 | | 3 | | 10 | | 8.222 | |
| AR | 7.738 | | 8.988 | | 7.967 | | 8.210 | | 4.122 | | 9.161 | | 1.999 | | 2.813 | | 8.422 | | 6.602 | |
| Ho | 0.697 | | 0.500 | | 0.500 | | 0.636 | | 0.250 | | 0.613 | | 0.097 | | 0.333 | | 0.455 | | 0.453 | |
| He | 0.770 | | 0.835 | | 0.792 | | 0.711 | | 0.548 | | 0.813 | | 0.297 | | 0.335 | | 0.820 | | 0.658 | |
| Fis | 0.096 | | **0.405** | | **0.373** | | 0.106 | | **0.548** | | **0.250** | | **0.677** | | 0.006 | | **0.449** | | **0.314** | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | *df* | Sum of squares | Variance components | Variation (%) | Fixation indices | *P* value |
| **AMOVA between shorelines** |  |  |  |  |  |  |
| Among groups | 1 | 11.013 | 0.00914 Va | 0.30 | FCT = 0.00296 | 0.098 |
| Among populations within groups | 12 | 82.046 | 0.05796 Vb | 1.88 | FSC = 0.01883 | 0.000 |
| Within populations | 918 | 2772.745 | 3.02042 Vc | 97.82 | FST = 0.02173 | 0.000 |
| Total | 931 | 2865.804 | 3.08752 |  |  |  |
|  |  |  |  |  |  |  |

**Table S5.** Analysis of molecular variance of the sites where *Ciona intestinalis* was collected. Sites were separated into different shorelines (Northern English Channel and Southern English Channel).



**Figure S1.** Gel electrophoresis after the mtCOI gene had been treated with *Rsa*I restriction enzyme. A and B represent *Ciona robusta* (sampled from Plymouth) *and Ciona intestinalis* (sampled from Town Quay)individuals respectively. In *C. robusta* individuals, the mtCOI gene has been digested by *Rsa*I leading to two clear bands; in *C. intestinalis* individuals, the mtCOI gene has not been digested, resulting in one band.

****

**B**

**Bb**

**Ab**

**Figure S2.** Population structure of: A) the seven ‘Northern’ sampling sites with K = 3, as inferred by STRUCTURE; B) the seven ‘Southern’ sampling sites (excluding Jersey) with K = 2, as inferred by STRUCTURE. Each individual is represented by a single bar, with the likelihood of membership to different clusters indicated by the colours. Bold lines separate sample sites, with site abbreviations below the plot. Abbreviations of site names as in Table 1.

****

**Figure S3.** Plots of the first two axes obtained by Discriminant Analysis of Principal Components. Labels are placed at the centre of each group, further delineated by inertia ellipses. Abbreviations of site names as in Table 1.

**Figure S4.** Correlation between geographic distance (km) and FST values (A) including Jersey and NOCS (r = 0.316, *P* = 0.008); (B) excluding Jersey and NOCS (r = 0.506, *P =* 0.005); (C) ‘Northern’ populations (r = 0.055, *P* = 0.349); (D) ‘Southern’ populations (r = 0.190, *P* = 0.123).

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