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

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Running head: Moving genotypes within native ranges

Abstract

Human activities are responsible for the translocation of vast amounts of organisms, altering natural patterns of dispersal and gene flow. Most research to date has focused on the consequences of anthropogenic transportation of non-indigenous species within introduced ranges, while little research has focused on native species.

Here we compared genetic patterns of the sessile marine invertebrate, *Ciona intestinalis*, which has highly restricted dispersal capabilities. We collected samples in a region of its native range where human activities that are known to facilitate spread are prevalent. Using microsatellite markers, we revealed highly dissimilar outcomes. Firstly, we found low levels of genetic differentiation among sites separated by both short and large geographic distances, indicating the presence of anthropogenic transport of genotypes, as well as little influence of natural geographic barriers or isolation-by-distance. Secondly, we found significant genetic differentiation in pairwise comparisons among certain sites, suggesting that other factors besides artificial transport (e.g. fine-scale natural dispersal) may be shaping genetic patterns. Taken together, we found dissimilar patterns of population structure in a highly urbanised native range that could not be predicted by artificial transport alone. We conclude that anthropogenic activities alter genetic composition of native ranges, with unknown consequences for species' evolutionary trajectories.

Keywords:

Larval transport, dispersal pathways, population connectivity, range shifts, tunicates.

Introduction

Identifying the magnitude and scale of connectivity among populations is fundamental for understanding the biogeography, ecology and evolutionary biology of species (1, 2). The degree to which natural populations are connected is often correlated to their dispersal capability, particularly for species with short propagule duration (3). Natural dispersal is geographically limited by the movement capabilities of adults and their propagules (i.e. juveniles, spores, seeds, or larvae). Thus, it is generally assumed that isolation-by-distance (IBD) models shape genetic variation patterns within native ranges.

With the onset of the Anthropocene the translocation of species beyond their native ranges has become commonplace, resulting in major alterations to natural population connectivity patterns (4). For instance, anthropogenic transport may nullify IBD patterns (e.g. no correlation between population structure and geographic distance) (5) and / or alter the genetic composition of populations (6, 7). Whilst most existing literature has focused on the effects of anthropogenic transport on introduced species (8-10), little is known about how anthropogenic transport affects marine species in their native ranges. This is especially the case for species inhabiting artificial habitats, where anthropogenic transport is intense and thus human-mediated connectivity expected.

Here we studied the genetic patterns of the marine invertebrate, *Ciona intestinalis* (Tunicata, Chordata), found in a highly urbanised native range. This tunicate has poor natural dispersal capabilities with pelagic larval duration below 24 h (11-13) and thus long-distance dispersal can only be achieved by anthropogenic means. We addressed two fundamental questions: (i) Does human mediated transport affect native genetic signatures in the same way as it

does in introduced ranges? (ii) Is there any relationship between native genetic composition and dispersal distance?

Materials and methods

We used as a model system the species *Ciona intestinalis*, which is native to the North Atlantic (14). It is mostly reported in artificial habitats and due to its fouling behaviour, it is prone to be transported by anthropogenic means (mainly via hull fouling) (see Appendix S1). We conducted samplings between June and December 2014 at fifteen sites (Table 1, Fig. 1, Appendix S2) where this species is widespread. Across the studied region, commercial shipping is intense (routes between sample sites have often more than 140 vessels per day, see www.marinetraffic.com for live maps of vessel tracking). The same holds for recreational shipping which connects distant marinas (for example see the Jersey marina in Table S3).

We extracted genomic DNA from each individual using the NucleoSpin 96 Tissue Kit (Machery-Nagel) following the manufacturer's protocol (Appendix S3) and amplified nine microsatellite loci by PCR (Table 2, Appendix S4, Table S1, S2). We calculated different pairwise population genetic differentiation measures and their significance (see details in Appendices S5). We then visualised population structure using a bayesian approach implemented in STRUCTURE v. 2.3.4 (15) and a discriminant analysis of principal components (DAPC) (see further details in Appendix S5). We performed a Mantel test (16) in order to ascertain the correlation between geographic distance (see Appendix S5) and genetic distance among the studied populations.

Results

We genotyped a total of 484 individuals (see details on genetic identification in Appendix S6 and Fig. S1, and genetic diversity in Appendix S7). Similar findings were observed with F_{ST} and D indices: 1. Non-significant differentiation among 49% and 42% of the pairwise population comparisons respectively; 2. Differentiation between both distant and close populations (Table 2, see also Appendix S8).

The STRUCTURE analysis clearly distinguished two genetic clusters, one that assigned most individuals from Jersey and NOCS (87% and 65% respectively), and the other that contained 84% - 99% of individuals from the other populations (Fig. 2A; see details in Appendix S9). The same was found for the DAPC analysis (Fig. 2B), in which the primary axis (x-axis) separated Jersey and NOCS from the rest of the locations (for further details see Appendix S9, Fig. S3).

We found a correlation between genetic and geographic distance both when all populations were included (Fig. S4A) and when the most genetically divergent populations were excluded (Fig. S4B). However, we found no correlation between genetic and geographic distance between sub-regional groups of populations (i.e. northern or southern populations) which would have been expected under the hypothesis of natural stepwise dispersal (Appendix S9, Figs. S4C, S4D).

Discussion

Within native ranges, high levels of population structure and genetic divergence is expected among geographically isolated populations (17), especially in species with poor dispersal abilities (18). Human-mediated transport can promote artificial connectivity that translates into high levels of

gene flow among populations that would otherwise have been genetically differentiated. This is clearly exemplified by studies investigating introduced ranges of non-indigenous species (6, 19, 20). Here we compared genetic patterns within the native range of the studied species where human activities that are known to facilitate spread are prevalent. The results revealed highly divergent outcomes in terms of genetic differentiation. Firstly, we found low levels of genetic differentiation among sites, indicating the presence of anthropogenic transport of genotypes, as well as little influence of natural geographic barriers or isolation-by-distance. Second, we found significant genetic differentiation in pairwise comparisons among certain sites, suggesting that other factors besides artificial transport (e.g. fine-scale natural dispersal) may be shaping genetic patterns. Thus, we found dissimilar patterns of population structure that could not be predicted based on geographic location, dispersal type / intensity or the homogenising effect of artificial transport.

In our study many pairwise population comparisons showed no significant genetic differentiation, including comparisons among distant sites (e.g. StQ and BTN, see Table 2). This is consistent with a growing number of studies showing how anthropogenic transport prevents drift of allele frequencies (21) and homogenises genotypic composition (18, 19, 22) within introduced ranges. Our results show evidence of artificial transport of genotypes that nullifies IBD patterns in similar ways as has been reported for non-indigenous species with similar natural dispersal abilities (e.g. (23)). The artificial transport of genotypes inevitably leads to alterations of species' evolutionary trajectories (e.g. disruption of local adaptation) with unforeseen consequences for species ranges.

Besides the above patterns of genetic homogeneity, we also found patterns of significant genetic differentiation among certain sites (Table 2). For

example, Jersey and NOCS exhibited high genetic differentiation compared to all other sites, irrespective of geographical distance (Table 2). These results are surprising considering the highly connected studied region. This shows how unpredictable genetic patterns can be in highly urbanised regions where human-mediated transport is prevalent. Although artificial dispersal is evidently the major driver shaping genetic composition of the studied region, other more inconspicuous factors may play an important role. For example, a complex interplay between natural and artificial dispersal patterns shaping fine-scale genetic signatures could be present, although further work in needed to clarify this.

A possible explanation for the weak patterns of genetic differentiation found among some sites is the presence of a large effective population size (24). However, our results showed heterogeneous patterns of genetic differentiation and at times very high differentiation among certain population pairwise comparisons, suggesting that large effective population size cannot alone explain the results.

Shipping data (Fig. 1 and Table S3) showed certain variability in terms of geographic links and shipping intensity but the overall pattern suggested high connectivity among all sampled sites. Indirect links via, for example, a stepping-stone model (25) as a result of newly built marine infrastructures could also contribute to enhancing dispersal within the native range (26). In addition, studying natural populations (i.e. not from marine infrastructures) is needed to discern between artificial and natural gene flow. However, this is challenging in the study species, as it is hard to find away from marine infrastructures. Overall, studies of urbanised and protected regions (e.g. 27) are key for discerning between natural and artificial dispersal, as well as ancestral and contemporary changes in genetic composition.

To conclude, we found evidence of connectivity patterns likely due to the artificial transport of genotypes within the studied native range but also significant genetic differentiation among sites. This is particularly well illustrated by: 1) patterns of genetic homogeneity among both close and distant sites and 2) highly dissimilar genetic composition when geographically close sites were compared. This result highlights the erratic nature of population connectivity as a result of artificial transport. We conclude that human-mediated transport severely alters evolutionary trajectories within native ranges through decreasing inbreeding depression and disrupting local adaptation patterns, with unknown consequences for the fate of both native and introduced species ranges.

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Ethical statement, including your project number

The research described here involved marine invertebrates, namely ascidians. We chose to work with marine invertebrates due to their importance in terms of marine bioinvasions worldwide and also as all the members of the proposal have extensive experience working with these animals. At no point will any experiments involving vertebrates be carried out. Collection of animals will never include protected or endangered species.

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Competing interest statement

We declare we have no competing interests.

Author contributions and conformity

The enclosed work submission for publication has been approved by all authors and institutions, and all persons entitled to authorship have been so named. All authors have seen and agreed to the submitted version of the manuscript. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Dryad or data accessibility statement

The generated data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.27fb8. See reference list (28).

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Appendices of the paper entitled:

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Appendix S1. Studied species

The vase tunicate, *Ciona intestinalis* (Linnaeus 1767) (Ascidiacea, Tunicata, Chordata), is a solitary hermaphroditic ascidian that inhabits shallow waters from temperate to boreal regions (Caputi et al. 2007). Populations of *C. intestinalis* inhabiting natural habitats are poorly known (Dybern 1965) 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 (Caputi et al. 2007; Iannelli et al. 2007; Procaccini et al. 2011; Satoh et al. 2003). Comparisons of genomic DNA from northern European and Pacific *C. intestinalis* individuals revealed the putative existence of cryptic diversity (Suzuki et al. 2005). 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) (Brunetti et al. 2015; Pennati et al. 2015). These studies concluded that *C. intestinalis* type A corresponds to *Ciona robusta* and *C. intestinalis* type B to *Ciona intestinalis sensu* Millar 1953 (Brunetti et al. 2015; Pennati et al. 2015; Sato et al. 2012). *Ciona robusta* is thought to be native to the north-western Pacific Ocean (Nydam, Harrison 2007), although it has been introduced to the eastern Pacific Ocean (Castilla, Neill 2009; Lambert, Lambert 1998), Europe (Caputi et al. 2007; Nydam, Harrison 2007, 2011) and the southern hemisphere (McDonald 2004; Rius et al. 2014). *Ciona intestinalis* is believed to be native to the northern Atlantic Ocean and has since presumably been introduced to the western Atlantic Ocean (Nydam, Harrison 2007). In the English Channel, *C. robusta* has been introduced within the native range of *C. intestinalis*, leading to an area of sustainable sympatry (Bouchemousse et al. 2015; Nydam, Harrison 2011). When invasive, *C. intestinalis* and *C. robusta* can cause serious economic damages to aquaculture and ecological changes due to negative effects on native assemblages (Blum et al. 2007; Lambert, Lambert 2003; Ramsay et al. 2008; Rius et al. 2011).

Ciona intestinalis produce motile, lecithotrophic larvae that remain in the plankton for one to six days before settlement (Svane, Havenhand 1993). This leads to a limited dispersal capability, with models predicting a maximum dispersal of *c.* 6 km (Collin et al. 2013; Kanary et al. 2011; Petersen, Svane 1995). 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 (NOCS), Isle of Wight, Jersey), included in temporal monitoring and sampling (i.e. South English Channel populations (Bouchemousse et al. 2015)) or had not been sampled since 2007 (Nydam, Harrison 2011). 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 three ropes.

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 (Selkoe, Toonen 2006). Microsatellites have been developed from *C. robusta* and utilised in previous studies on *Ciona intestinalis* and *Ciona robusta* (Procaccini et al. 2000; Zhan et al. 2012; Zhan et al. 2010). However, obtaining reliable data using these microsatellites has since proven difficult because of the large evolutionary divergence between the two species (ca. 14%; Roux et al. 2013) 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; primers available upon request to F. Viard), and Cin12 (Zhan et al. 2010) 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 $MgCl_2$ (25 mM), 1.5 µL BSA (1 mg/ml), 0.1 µL Taq polymerase (Promega), 3.075 – 5.100 µL H_2O, 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 (Goudet 2001). We used Genepop On The Web v. 4.2 (Raymond, Rousset 1995) to estimate observed (H_O) and expected (H_E) 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 (Peakall, Smouse 2006, 2012). We examined deviations from Hardy-Weinberg equilibrium (HWE) using the Genepop On The Web v. 4.2 (Raymond, Rousset 1995) to compute the fixation index (F_{IS}) with a test based on a permutation procedure using 10 000 bootstrap replicates.

We assessed pairwise population genetic differentiation using F_{ST} values (Weir, Cockerham 1984) and their *P* values by running 10,000 permutations with Arlequin v.3.5.1.3 (Excoffier, Lischer 2010b). We also assessed the partition of genetic variance across populations using Jost's *D* (Jost 2008). F_{ST} has previously been used to measure genetic differentiation between populations, however over the past 10 years this measure has come under criticism (Hedrick 2005; Jost 2008). In the case of loci with two alleles, *D* and F_{ST} give the same estimate (Meirmans, Hedrick 2011). But when using populations with more than two alleles, F_{ST} has been criticised because the relative role of mutation and migration becomes a key issue (Hedrick 2005; Jost 2008). 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 (Balloux et al. 2000). As a result, Jost (2008) 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 F_{ST} (Whitlock 2011). For these reasons, both indices are included in the study. We used the package DEMEtics (Gerlach et al. 2010) in R (R Development Core Team, 2013) to calculate *D* values and their *P* values. If all the populations are in HWE, DEMEtics randomises 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) (Goudet et al. 1996). We obtained corrected *P* values of F_{ST} and *D* values using the Benjamini-Yuketieli method for multiple comparisons (Narum 2006).

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 (Raymond, Rousset 1995) as in Rousset (1997).

We conducted a hierarchical analysis of molecular variance (AMOVA) using Arlequin (Excoffier, Lischer 2010a) 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 used STRUCTURE v. 2.3.4 (Pritchard et al. 2000), 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 (Hubisz et al. 2009). 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 (Evanno et al. 2005). 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 (2013). 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 (Jombart et al. 2010). We used the package *adegenet* 1.4-2 (Jombart 2008) for R (R Development Core Team, 2013) 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 (Jombart et al. 2010). 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 (Jombart et al. 2010). DA, however, suffers limitations in that the number of variables (alleles) must be less than the number of observations (individuals) (Jombart et al. 2010). This is often not the case with highly polymorphic markers, and DA is hindered by correlations between variables (Jombart et al. 2010). 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 (Jombart et al. 2010).

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*". This taxonomic revision came from both morphological and molecular evidences, in particular diagnostic morphological characteristics were defined (2015; Pennati et al. 2015). These traits are however not always easy to observe during sampling in the field. For each specimen collected, we thus first confirmed the species identification made in the field by using a diagnostic mitochondrial molecular marker as detailed in Nydam & Harrison (2010) and Bouchemousse *et al.* (2015). Screening via gel electrophoresis (Fig. S1) of the digested mtCOI gene by *Rsa*I confirmed all samples analysed were *C. intestinalis*.

Appendix S7. Detailed results of the genetic diversity analyses

The generated data are available from 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 F_{IS} 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 F_{IS} 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* [H_E = 0.775 – 0.871, average 0.819 (Zhan et al. 2010); H_E = 0.510 – 0.875, average 0.760 (Zhan et al. 2012)]. As Zhan et al. (2010) identified, these findings were consistent with previous studies on individuals of the genus *Ciona*, which also described high levels of genetic diversity (Caputi et al. 2008; Kano 2007; Roux et al. 2013). Indeed, *C. intestinalis'* congener, *Ciona savignyi*, has been described as exhibiting 'the highest structural polymorphism ever comprehensively quantified in a multicellular organism' (Ilut et al. 2014; Small et al. 2007). The expected heterozygosities detected in this study were higher than those in other similar taxa including *Styela clava* [0.449 < H_E < 0.626 (Dupont et al. 2010; Dupont et al. 2009; Goldstien et al. 2010)], *Pyura chilensis* [0.219 < H_E < 0.298 (Haye, Muñoz-Herrera 2013)], and *Corella eumyota* [0.13 (Dupont et al. 2007b)].

All of the studied sites displayed significant heterozygote deficiencies (F_{IS} = 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 (Dakin, Avise 2004)] or non-random mating between individuals due to biological factors including inbreeding and Wahlund effect (Wahlund 1928). 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 (Dakin, Avise 2004), differential amplification of size-variant alleles (whereby smaller alleles are amplified more readily than larger alleles) (Wattier et al. 1998), and low or inconsistent DNA template quality (Dakin, Avise 2004). Null alleles can seemingly reduce genetic diversity within populations and therefore lead to erroneous results of heterozygote deficiency. They can also overestimate F_{ST} under some circumstances, for instance when gene flow is limited (Chapuis, Estoup 2007). 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. (2010). 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 (Petersen, Svane 1995; Svane, Havenhand 1993). 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) (Petersen, Svane 1995; Svane, Havenhand 1993). 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 (Svane, Havenhand 1993). 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 (Havenhand, Svane 1991). 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 (Dupont et al. 2009; Zhan et al. 2010). Bouchemousse et al. (2015) showed less than 5% of fertilization success in self-crosses. Inbreeding (selfing being an extreme form of 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 (Dupont et al. 2009; Hartl, Clark 1997). For these reasons, the significant high values of F_{IS} 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 (Duran et al. 2004). 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 (Dupont et al. 2007a).

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, Aber Wrac'h, and Saint-Quay-Portrieux had a higher percentage of individuals assigned to one cluster (green in Fig. S2B) while Perros-Guirec and Saint-Malo had the majority of individuals belonging to the other cluster (red in Fig. S2B). Individuals from Camaret and Roscoff 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 (for example the common shore crab, *Carcinus maenus* (Maes, Volckaert 2002; Roman, Palumbi 2004). Our study additionally proposes that different types of vectors influence the outcome of translocations. Murray *et al.* (2011) 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 (Carlton, Geller 1993).

The presence of private alleles is generally attributed to the existence of isolation among populations and/or recent species expansions (Chaves-Fonnegra et al. 2015; Duran et al. 2004; Slatkin 1985). Whilst private alleles have been suggested to correlate with genetic structure (Slatkin 1985), this has only been validated using simple models such as stepwise mutation model (Szpiech, Rosenberg 2011). 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 (Hohenlohe 2004). Accordingly, the limited natural dispersal capability of *C. intestinalis* (Collin et al. 2013; Kanary et al. 2011; Petersen, Svane 1995) 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%) F_{ST} values (Table 2)]. Weak pattern of genetic differentiation can be produced with limited gene flow when species display large effective population size (Gagnaire et al. 2015), 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 (Ménesguen, Gohin 2006), 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 (Kenchington et al. 2006)]. 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 (Bilton et al. 2002; Zhan et al. 2009). 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 (Collin et al. 2013). 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 (Collin et al. 2013). 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 (Lacoursière-Roussel et al. 2012) partly due to the unregulated nature of recreational boating hull fouling (Murray et al. 2011). 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 F_{ST} 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 (2016a) and Jersey to Poole (2016b). 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 (Ramsay et al. 2008). 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 (Ramsay et al. 2008). 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 (MMO 2014). 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 F_{ST} 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 F_{ST} 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 (2006)]. 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' (Waples, Gaggiotti 2006). The configuration of sample sites can limit gene flow between populations, even in the presence human-mediated transport (Dupont et al. 2009; Zhan et al. 2012). 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 (N° 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	(Zhan et al. 2010)

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.

Location	Year				
	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 different populations. 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 (H_O) and expected (H_E) heterozygosities; and fixation index (F_{IS}). Significant F_{IS} values are in bold. Means over loci (or global value for F_{IS}) 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 (NOC)										
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**

Table S5. Analysis of molecular variance for *Ciona intestinalis*. Sites were separated into different shorelines (Northern English Channel and Southern English Channel).

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

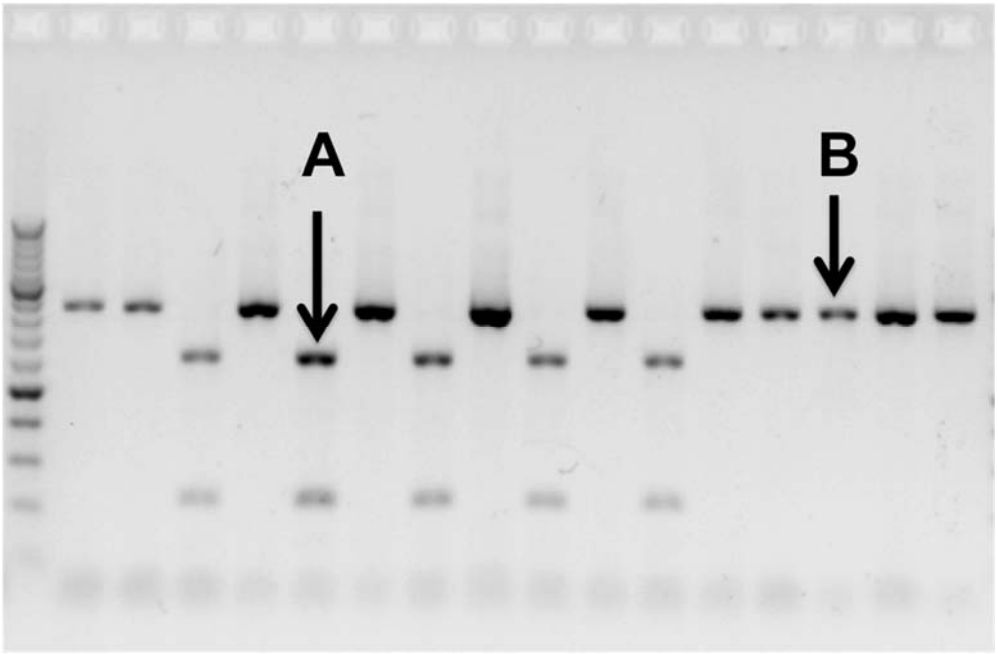


Figure S1. Gel electrophoresis after the mtCOI gene had been treated with *Rsa*I restriction enzyme. A and B represent *Ciona robusta* (sampled from Plymouth) *and Ciona intestinalis* (sampled from Town Quay) individuals respectively. In *C. robusta* individuals, the mtCOI gene has been digested by *Rsa*I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

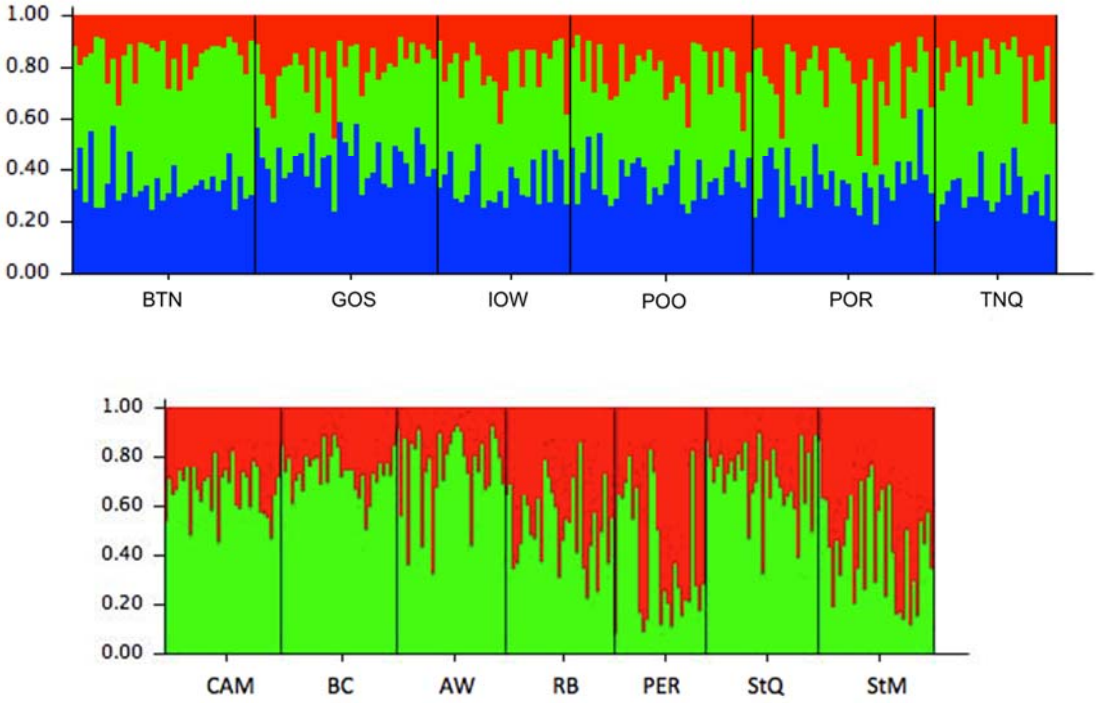


Figure S2. Population structure at: A) the six 'Northern' sampling sites with K = 3, as inferred by STRUCTURE; B) the seven 'Southern' sampling sites (excluding Jersey) with K = 2, as inferred by STRUCTURE. Each individual is represented by a single bar, with the likelihood of membership to different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

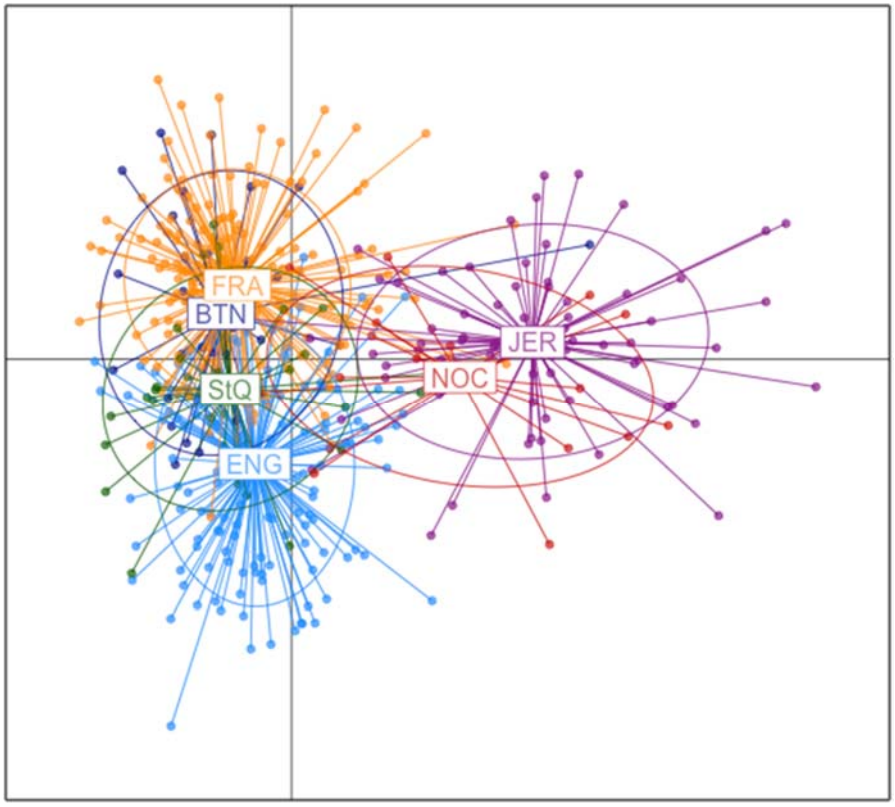
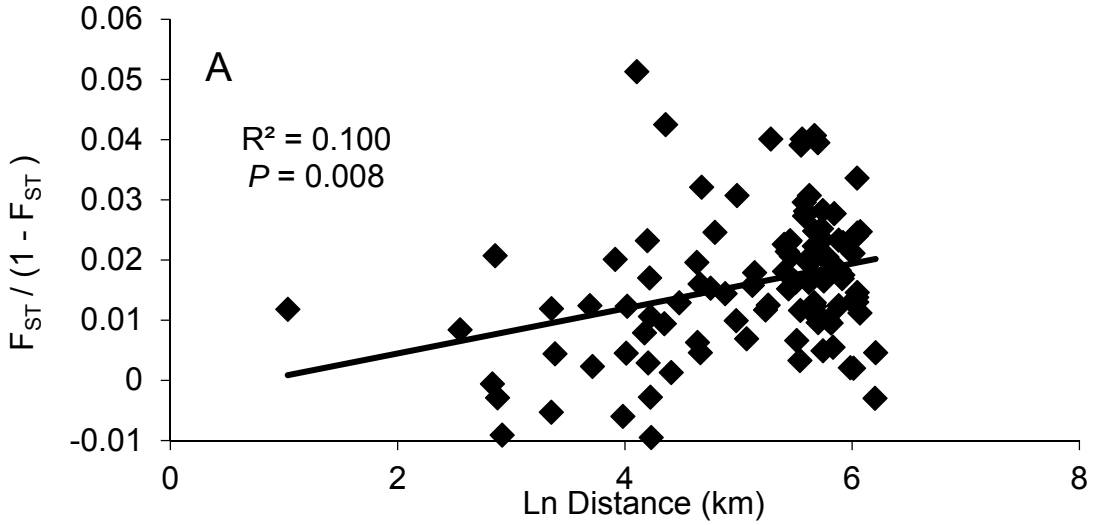
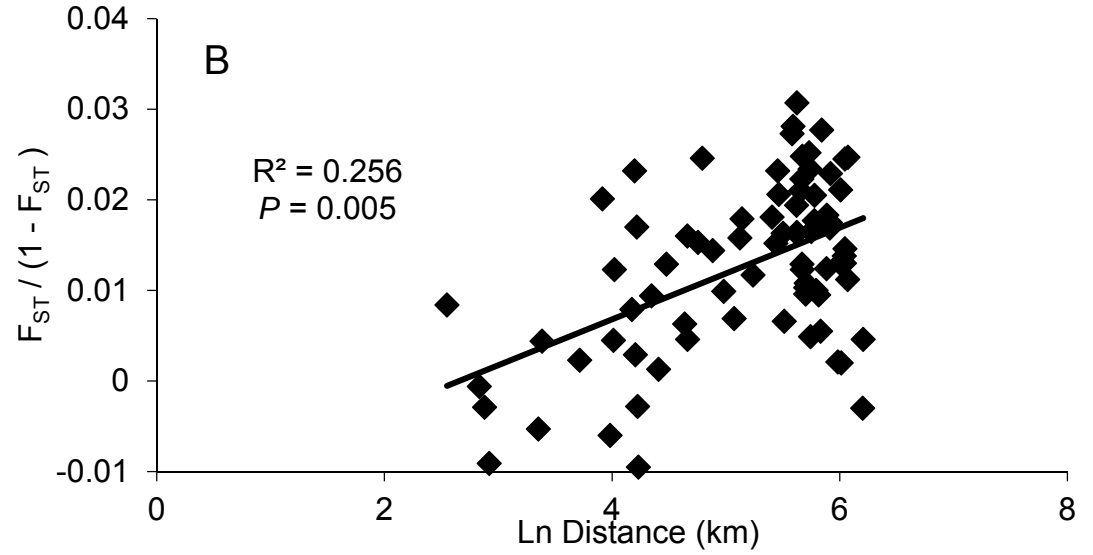


Figure S3. Plots of the first two axes obtained by Discriminant Analysis of Principal Components. Labels are placed at the centre of each group, further delineated by inertia ellipses. BTN = Brighton; StQ = Saint-Quay-Portrieux; FRA = Camaret, Brest, Aber Wrac'h, Roscoff, Perros-Guirec, and Saint-Malo; JER = Jersey; NOC = NOCS; and ENG = Gosport, Isle of Wight, Poole, Portsmouth, and Town Quay.


A
R² = 0.100
P = 0.008
0.06
0.05
0.04
0.03
0.02
0.01
0
-0.01
0
2
4
6
8
Ln Distance (km)
F_ST / (1 - F_ST)


B
R² = 0.256
P = 0.005
0.04
0.03
0.02
0.01
0
-0.01
0
2
4
6
8
Ln Distance (km)
F_ST / (1 - F_ST)

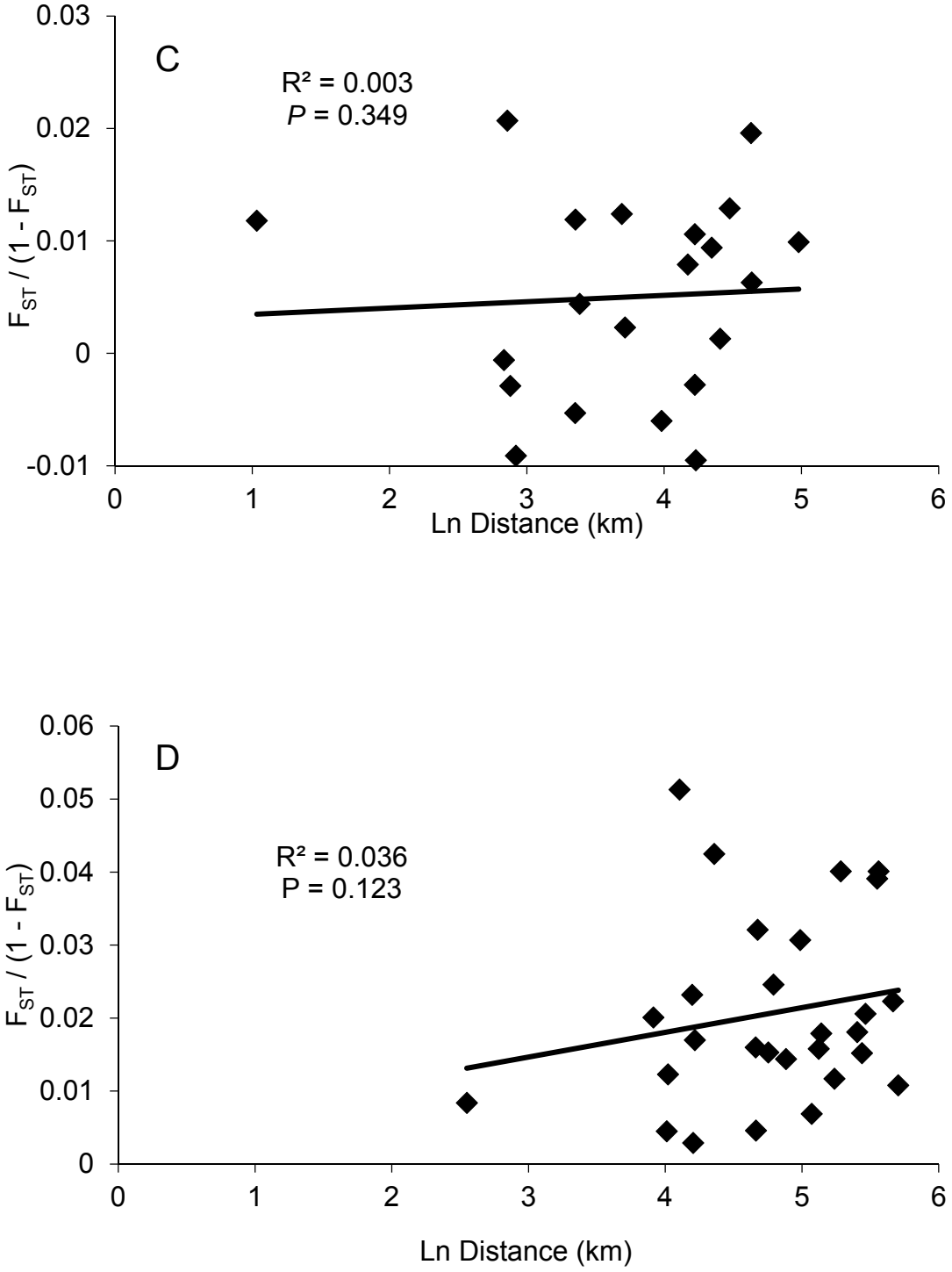


Figure S4. Correlation between geographic distance (km) and F_{ST} values (A) including Jersey and NOCS (r = 0.316, *P* = 0.008); (B) excluding Jersey and NOCS (r = 0.506, *P* = 0.005); (C) 'Northern' populations (r = 0.055, *P* = 0.349); (D) 'Southern' populations (r = 0.190, *P* = 0.123).

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