# 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, with little research focusing on native species.

Here, we compared genetic patterns of the sessile marine invertebrate, *Ciona intestinalis*, which has highly restricted dispersal capabilities. We collected individuals in a region of the species’ native range where human activities that are known to facilitate the artificial spread of species are prevalent. Using microsatellite markers, we revealed highly dissimilar outcomes. First, we found low levels of genetic differentiation among sites separated by both short and large geographical distances, indicating the presence of anthropogenic transport of genotypes, and little influence of natural geographical barriers. Second, we found significant genetic differentiation in pairwise comparisons among certain sites, suggesting that other factors besides artificial transport (e.g. natural dispersal, premodern population structure) may be shaping genetic patterns. Taken together, we found dissimilar patterns of population structure in a highly urbanized region 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 history of species [1,2]. The degree to which natural populations are connected is often correlated to their dispersal capability [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 artificial 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 geographical distance) [5] and/or alter the genetic composition of populations [6,7]. While most existing literature has focused on the effects of artificial 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 human-mediated transport is intense and thus artificial connectivity expected.

Here, we studied the genetic patterns of the marine invertebrate, *Ciona intestinalis* (Tunicata, Chordata), found in a highly urbanized 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

The species *C. intestinalis* is native to the North Atlantic [14] and is mostly reported in artificial habitats. Due to its fouling behaviour, this species is prone to be transported by anthropogenic means (mainly via hull fouling; see electronic supplementary material, appendix S1). We conducted samplings between June and December 2014 at 15 sites (table 1 and figure 1; electronic supplementary material, appendix S2) where this species is widespread. Across the studied region, commercial shipping is intense (routes between the sampled sites often have more than 140 vessels per day; see www.marinetraffic.com for live maps of vessel tracking). The same holds for recreational shipping that connects distant marinas (see for example details on shipping traffic of the Jersey marina in the electronic supplementary material, table S3).

We extracted genomic DNA from each individual using the NucleoSpin 96 Tissue Kit (Machery-Nagel) following the manufacturer’s protocol (see the electronic supplementary material, appendix S3) and amplified nine microsatellite loci by PCR (table 2; electronic supplementary material, appendix S4 and tables S1 and S2). We calculated different pairwise population genetic differentiation measures and their significance (see details in the electronic supplementary material, appendices S5). We then visualized population structure using STRUCTURE v. 2.3.4 [15] and a discriminant analysis of principal components (DAPC; see further details in the electronic supplementary material, appendix S5). We then performed a Mantel test [16] in order to ascertain the correlation between geographical and genetic distances (see the electronic supplementary material, appendix S5) among the studied sites.

# Results

We genotyped a total of 484 individuals (table 1, see details on genetic identification in the electronic supplementary material, appendix S6 and figure S1, and genetic diversity in the electronic supplementary material, appendix S7). Similar findings were observed with FST 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 the electronic supplementary material, 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 sites (figure 2a; see details in the electronic supplementary material, appendix S9). The same was found for the DAPC analysis (figure 2b), in which the primary axis (x-axis) separated Jersey and NOCS from the rest of the sites (for further details, see the electronic supplementary material, appendix S9, and figure S3).

We found a correlation between genetic and geographical distance both when all sites were included (electronic supplementary material, figure S4a) and when the most genetically divergent sites were excluded (electronic supplementary material, figure S4b). However, we found no correlation between genetic and geographical 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 (electronic supplementary material, appendix S9 and figures S4c,d).

**Discussion**

Within native ranges, high levels of population structure and genetic divergence are 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 be genetically differentiated. This is clearly exemplified by studies investigating introduced ranges of non-indigenous species [6,19,20]. Here, we compared genetic patterns in a region of the native range where human activities that are known to facilitate spread are prevalent. The results revealed highly divergent outcomes in terms of genetic differentiation. First, we found low levels of genetic differentiation among sites, indicating the presence of anthropogenic transport of genotypes, as well as little influence of natural geographical barriers or IBD. Second, we found significant genetic differentiation in pairwise comparisons among certain sites, suggesting that other factors besides artificial transport (e.g. natural dispersal, premodern population structure) may have shaped genetic patterns. Thus, we found dissimilar patterns of population structure that could not be predicted based on geographical location, dispersal type/intensity or the homogenizing 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 homogenizes genotypic composition [18,19,22] within introduced ranges. Our results show evidence of artificial transport of genotypes in ways similar to what has been reported for non-indigenous species with similar natural dispersal abilities (e.g. [23]). The artificial transport of species inevitably leads to alterations of 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 with 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 urbanized native ranges. 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 is 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 (see the electronic supplementary material, table S3) showed certain variability in terms of geographical 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 in both native and introduced ranges [26,27]. In addition, studying natural populations (i.e. not from marine infrastructures) is key to discern between artificial and natural gene flow. However, this is challenging in the studied species, as *C. intestinalis* is hard to find away from marine infrastructures. Overall, studies of urbanized regions 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 artificial transport of genotypes within the studied native range but also significant genetic differentiation among sites. This is particularly well illustrated by: (i) patterns of genetic homogeneity among both close and distant sites and (ii) highly dissimilar genetic composition when geographically close sites were compared. This result highlights the erratic nature of population connectivity in highly urbanized regions. We conclude that human-mediated transport severely alters evolutionary trajectories within native ranges through decreasing inbreeding depression and disrupting local adaptation patterns. This is an unprecedented form of global change that has unknown consequences for the fate of both native and introduced species ranges.

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**Table and Figure captions**

**Table 1.** Sampling information of *Ciona intestinalis* including site name; population abbreviations (code); geographical location; numbers of individuals analysed; and the date individuals were collected.

**Table 2**. Measures of genetic differentiation in pairwise comparisons of the studied sites. Jost’s D values are shown above the diagonal and FST values below the diagonal. Significant values are in italics after the Benjamini–Yuketieli method for multiple comparisons. Abbreviations are as in table 1.

**Figure 1.** Map of sampled sites of *Ciona intestinalis*. Site names are abbreviated as in Table 1.

**Figure 2.** a) Population structure at the 15 sampling sites with K = 2, as inferred by STRUCTURE. B) Plot of the first two axes obtained by Discriminant Analysis of Principal Components on the sample sites as described in methods. Abbreviations as in table 1. Labels are placed at the centre of each population, further delineated by inertia ellipses.