The Impact of Yangtze River Discharge, Ocean Currents and Historical Events on the Biogeographic Pattern of *Cellana toreuma* along the China Coast

Yun-wei Dong¹,², Hai-shan Wang¹, Guo-Dong Han¹, Cai-huan Ke¹, Xin Zhan¹, Tomoyuki Nakano³, Gray A. Williams⁴

¹ State Key Laboratory of Marine Environmental Science, College of Oceanography and Earth Science, Xiamen University, Xiamen, China, ² Marine Biodiversity and Global Change Laboratory, Xiamen University, Xiamen, China, ³ Seto Marine Biological Laboratory, Field Science Education and Research Centre, Kyoto University, Nishimuro, Wakayama, Japan, ⁴ The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China

Abstract

**Aim:** Genetic data were used to measure the phylogeographic distribution of the limpet, *Cellana toreuma* along the China coast in order to ascertain impacts of historic events, ocean currents and especially freshwater discharge from the Yangtze River on the connectivity of intertidal species with limited larval dispersal capability.

**Methodology/Principal Findings:** Genetic variation in 15 populations of *C. toreuma* (n = 418), ranging from the Yellow Sea (YS), East China Sea (ECS) and South China Sea (SCS), were determined from partial mitochondrial cytochrome c oxidase subunit I gene. Genetic diversity and divergence based on haplotype frequencies were analyzed using CONTRIB, and AMOVA was used to examine genetic population structure. Historic demographic expansions were evaluated from both neutrality tests and mismatch distribution tests. Among the 30 haplotypes identified, a dominant haplotype No. 1 (H1) existed in all the populations, and a relatively abundant private haplotype (H2) in YS. Pairwise FST values between YS and the other two groups were relatively high and the percentage of variation among groups was 10.9%.

**Conclusions:** The high nucleotide and gene diversity in the YS, with large pairwise genetic distances and relatively high percentages of variation among groups, suggests that this group was relatively isolated from ECS and SCS. This is likely driven by historic events, ocean currents, and demographic expansion. We propose that freshwater discharge from the Yangtze River, which may act as physical barrier limiting the southward dispersal of larvae from northern populations, is especially important in determining the separation of the YS group from the rest of the Chinese populations of *C. toreuma*.

Citation: Dong Y-w, Wang H-s, Han G-D, Ke C-h, Zhan X, et al. (2012) The Impact of Yangtze River Discharge, Ocean Currents and Historical Events on the Biogeographic Pattern of *Cellana toreuma* along the China Coast. PLoS ONE 7(4): e36178. doi:10.1371/journal.pone.0036178

Copyright: © 2012 Dong et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by grants from Natural Science Foundation for National Natural Science Foundation of China (41076083), Distinguished Young Scholars of Fujian Province, China (2011J06017) and the Fundamental Research Funds for the Central Universities (201012028). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: dongyw@xmu.edu.cn

Introduction

Understanding the forces which contribute to the biogeographic distributions of marine organisms has long been of interest to biologists in their attempts to interpret present day patterns of marine biodiversity [1,2]. Species in the intertidal zone are excellent candidates to study the influence of various factors on biogeographic distribution as they occupy a discrete, narrow strip of habitat which is linked by the sea [3], and so it is relatively easy to identify the factors which affect their distribution. The distributions of intertidal species are closely linked to historic events, affecting the connectivity of different regions [4–7], present day coastal topography and oceanic currents which affect larval supply [8–11,12,13], species’ ecological requirements [7,14] and also present day changes in climate which influence the on-shore success of populations [15–17].

Fossil and pollen records have shown that historical climate change is a pivotal factor affecting the abundance and distribution of species [18,19], and genetic data also confirm that historic events have had a significant impact on the distribution of populations [20–24], especially during the last glacial maximum (LGM) [18,25,26]. Previous studies have, for example, shown that the LGM did not extirpate the majority of species in the northeastern Pacific; instead, many species persisted in regional glacial refuges during the LGM, and this appears to be a common biogeographic history for many rocky-shore organisms in this region [6,7].

As many intertidal species have planktonic larvae which can be carried in the water column before settlement for days or weeks [27–29], this larval dispersal phase is another important regional process affecting the distribution of intertidal species [30–32]. The dispersal distances of many marine organisms are directly related to the time that larvae spend in the plankton [29], however, the pelagic larval durations (PLD) among organisms are species, season and location specific, and dispersal distance is closely...
related to local and regional hydrographic conditions, biological characteristics [11], and habitat specificity [33,34].

Limpets are important keystone grazers on rocky shores [35–37] and historic events have been shown to play a critical role in structuring the gene pool of patellid limpets from the temperate Northern Hemisphere [38–41]. Historic events have also been implicated as the main driver to explain the biogeography of nacellid limpets in New Zealand, with the formation of a geographic barrier by the Cook Strait being important for the current day biogeographic patterns observed in this group [23,42,43]. Maintenance of population connectivity through larval dispersal has also been shown to be important, the main ocean currents flowing along the Atlantic coast of Iberia have, for example, been responsible for maintaining connectivity of populations of _Patella rustica_ along the coast through occasional larval transport [44]. Therefore, the complicated biogeographic patterns of limpets can serve as a model for understanding the present day distribution patterns of intertidal species along heterogeneous coastlines.

The distribution patterns of intertidal species along the coast of China are poorly studied but are likely to be influenced by past historic events as well as present day environmental conditions. The Bo, Yellow and East China Sea along the continental shelf of China, for example, are shallow seas and when global sea levels fell during the Quaternary glaciations, these three seas are thought to have become partly continuous landmasses (Fig. 1). Sea surface temperatures (SST) also decreased during this period [45] and such environmental changes are suggested to have affected species’ distributions [46–50]. Besides historical events, ocean currents also play an important role in population connectivity along the China coast. The biogeography of a high shore barnacle, _Hexechamaesipho pilshyri_ (Hiro, 1936), for example, has been shown to be strongly influenced by the Kuroshio Current [47]. Hydrographic patterns can also be affected by outflows from major rivers [51]. Flow from the Yangtze River, the third largest river in the world with an average annual discharge of 8–9×10**11** m**3** [52], can influence surrounding ocean currents [53], and may subsequently limit dispersal of planktonic larvae and act as a barrier for genetic connectivity of intertidal species, and hence play an important role in the biogeographic pattern of intertidal communities.

The limpet _Cellana toreuma_ (Reeve, 1855) is widely distributed along the China coast [54], which makes it an ideal species to investigate the relative roles of historic events and present day environmental conditions on the past and present genetic connectivities of intertidal species in this region. Specifically, phylogenetic studies of 15 populations of _C. toreuma_ from Qingdao in the north, to Weizhoudao in the south of China were used to investigate the importance of historic events, the role of ocean currents and Yangtze River discharge in affecting the present day genetic connectivity of intertidal, rocky shores species along the China coast.
collections from public access areas, no specific permits were required to collect these specimens. They were divided into three groups, the Yellow Sea group (YS group, including QD and ZS), the East China Sea group (ECS group, including SS, ZS, and PT), and the South China Sea group (SCS group, including DS, ZS, and XM). As *C. toreuma* is not a protected species, collections were only made from public access areas, no specific permits were required to collect these specimens from these locations.

**Materials and Methods**

**Collections**

A total of 418 individuals of *Cellana toreuma* from 15 rocky shore localities which represented populations from different marginal seas were collected in 2009–2010 within a geographic range of ~2,600 km along the coastline of China (Fig. 1 and Table 1). The summer ocean currents along China coast and sea surface salinity in Yangtze River Estuary in summer [55] were shown in Fig. 1. Based on their locations, the 15 populations could be divided into three groups, the Yellow Sea group (YS group, including QD and DGD), the East China Sea group (ECS group, including SS, ZS, NJ, PT, NRD, MZD, CW, XM, DS, and NA) and the South China Sea group (SCS group, including SZ, HK, and WZD). As *C. toreuma* is not a protected species, and collections were only made from public access areas, no specific permits were required to collect these specimens from these locations.

**DNA extraction, PCR and sequencing**

All individuals were stored in absolute ethanol until DNA extraction. Genomic DNA was extracted from foot muscle tissue using methods as described in previous studies [56] with minor revisions. About 20 mg of foot muscle was dissected from each individual and homogenized in 400 μl lysis buffer (400 mM NaCl, 10 mM Tris-HCl (pH 8.0), 2 mM EDTA (pH 8.0), 1% SDS (w/v), 10 μl (10 mg ml⁻¹) of protease K was added to the homogenate, which was then incubated in a water bath at 55°C. After 1–3 h incubation, 400 μl NaCl (6 M) was added and the homogenate was centrifuged at 12,000 g for 30 min. An equal volume of isopropanol was added to the supernatant and then the mixture was centrifuged at 12,000 g for 15 min. The precipitate was washed using 70% ethanol twice and then dissolved in ultrapure water. A 598 nucleotide fragment of cytochrome oxidase subunit I mtDNA (COI) was amplified and sequenced using the following primers: LCO1490F5'-GGT CAA CAA ATC ATA AAG ATA TTG G -3', HCO2198R 5'-TAA ACT TCA GGG TGA CGA AAA AAT CA -3' [57].

**Data analyses**

Sequences of each individual were aligned with CLUSTAL X1.81 [58] and individual consensus sequences were retrieved with both alignment and manual checks. The accuracy of COI sequences was confirmed by translating the nucleotide data to amino acid sequences. Estimates of molecular genetic diversity (π, nucleotide diversity; H, gene diversity; FST values, and haplotype frequencies) were performed using ARLEQUIN 3.5 [59]. As sample sizes varied between different populations and groups, the programme CONTRIB 1.02 was used to standardized samples sizes, and haplotype frequencies were then calculated to estimate gene diversity and divergence of populations and groups [60]. Analysis of molecular variation (AMOVA) [61], performed in ARLEQUIN, was used to examine population genetic structure. Historical demographic expansions were examined by Tajima's D test [62] and Fu's Fs [63]. Neighbor-joining (NJ) tree was reconstructed using evolutionary distances computed with the Maximum Composite Likelihood method using the Tamura-Nei substitution model. To examine the relationship between genetic and geographic distance, the pairwise values of FST were calculated against geographic distance between all locations. The strength and significance of the relationship between genetic differentiation and geographic

**Table 1. Collection sites, sample size, allocated geographic group and summary of molecular diversity for *Cellana toreuma* collected along the coast of China.**

| ID  | Site         | Sample size | No. of haplotypes | Nucleotide diversity (π, Mean ± S.D.) | Gene diversity (%) Mean ± S.D. | Group |
|-----|--------------|-------------|-------------------|--------------------------------------|---------------------------------|-------|
| QD  | Qingshao (36°07'N, 120°36'E) | 21          | 3 (71% 23%)       | 0.00080 ± 0.00081                     | 0.45 ± 0.10                      | YS    |
| DGD | Dagongdao (36°02'N, 120°13'E) | 30          | 5 (67% 23%)       | 0.00096 ± 0.00089                     | 0.51 ± 0.08                      | YS    |
| SS  | Shengshan (30°07'N 121°55'E)   | 15          | 3 (86%)           | 0.00022 ± 0.00004                     | 0.26 ± 0.14                      | ECS   |
| ZS  | Zhoushan (29°57'N 122°12'E)    | 52          | 5 (90%)           | 0.00032 ± 0.00004                     | 0.18 ± 0.07                      | ECS   |
| NJ  | Nanjiliedao (27°33'N 120°41'E) | 30          | 5 (86%)           | 0.00056 ± 0.00064                     | 0.25 ± 0.10                      | ECS   |
| PT  | Pingtan (25°27'N 119°48'E)     | 29          | 3 (93%)           | 0.00023 ± 0.00039                     | 0.14 ± 0.08                      | ECS   |
| NRD | Nanruida (25°14'N 119°27'E)    | 32          | 4 (90%)           | 0.00032 ± 0.00046                     | 0.18 ± 0.09                      | ECS   |
| MZD | Meizhoudao (25°14'N 119°09'E)  | 24          | 4 (87%)           | 0.00028 ± 0.00044                     | 0.24 ± 0.11                      | ECS   |
| CW  | Chongwu (24°53'N 118°56'E)     | 32          | 4 (91%)           | 0.00032 ± 0.00046                     | 0.18 ± 0.09                      | ECS   |
| XM  | Xiamen (24°25'N 118°08'E)      | 32          | 5 (87%)           | 0.00042 ± 0.00054                     | 0.25 ± 0.10                      | ECS   |
| DS  | Dongshan (23°43'N 117°32'E)    | 32          | 2 (97%)           | 0.00011 ± 0.00026                     | 0.06 ± 0.06                      | ECS   |
| NA  | Nanao (23°26'N 117°00'E)       | 30          | 4 (90%)           | 0.00034 ± 0.00048                     | 0.19 ± 0.10                      | ECS   |
| SZ  | Shenzhen (22°32'N 114°13'E)    | 14          | 2 (86% 14%**)     | 0.00044 ± 0.00058                     | 0.26 ± 0.14                      | SCS   |
| HK  | Hong Kong (22°17'N 144°10'E)   | 31          | 3 (90%)           | 0.00032 ± 0.00046                     | 0.19 ± 0.09                      | SCS   |
| WZD | Weizhoudao (21°26'N 109°03'E)  | 14          | 2 (92%)           | 0.00048 ± 0.00061                     | 0.14 ± 0.12                      | SCS   |

1Values in the parentheses are the percentages of haplotype 1 in the populations; 2percentages of haplotype no. 10 in QD and DGD populations; 3Based on the geographical locations, these populations were divided into three groups. YS, ECS and SCS represent populations from Yellow Sea, East China Sea and South China Sea, respectively.

doi:10.1371/journal.pone.0036178.t001

**Phylogeography of Cellana toreuma**
distances were assessed with Mantel tests using IBD 1.53 [65]. To visualize the relationship between genetic and geographic distances, pairwise population $F_{ST}$ values were plotted against geographic distance using Ptraiss v5.0 (Graphpad Software, San Diego, CA, USA).

Results

Sequence variations

A 597-bp portion of COI was sequenced from 418 individuals of *Cellana toreuma* (GenBank access numbers JQ813140–JQ813557). Among all the individuals, 30 nucleotide sites were polymorphic. The genetic variation of COI mtDNA in the 15 populations of *Cellana toreuma* along the China coast was low, whether measured as gene diversity ($\theta = 0.087$, Table 2). In DGD ($DHs = 0.362$; $DHt = 0.404$) and QD ($DHs = 0.333$; $DHt = 0.087$, Table 2).

Genetic relationship among haplotypes

Of the 418 individuals from 15 populations, 30 haplotypes were present (see Appendix S1). A dominant haplotype No. 1 ($H1$) was found in all the populations (ca. 87% of individuals). The percentage of $H1$ in all populations ranged from 67.97%. In populations from the East China Sea, percentages of $H1$ in all the samples were $>85%$ (86%–97%). All other haplotypes differed from $H1$ by only one or two mutations. In the two populations from the Yellow Sea (QD and DGD), however, the percentages of haplotype No. 2 ($H2$) were both 23%, indicating $H2$ was an important private haplotype in these populations (Table 1, Fig. 2).

| Population levels | Samplesize | Nb Hap | $h_s$ | $r(13)$ | $DHs$ | $DHt$ | $DGst$ | $C_t$ | $C_s$ | $C_d$ | $C_t'$ | $C_s'$ | $C_d'$ |
|-------------------|------------|--------|-------|---------|-------|-------|--------|-------|-------|-------|--------|--------|--------|
| CW                | 32         | 4      | 0.181 | 0.090  | 1.219 | 0.207 | 0.211  | 0.018 | -0.017 | -0.014 | -0.003 | -0.111 | -0.004 |
| DG                | 30         | 5      | 0.515 | 0.089  | 2.290 | 0.362 | 0.404  | 0.104 | 0.108  | 0.086  | 0.022  | -0.008 | 0.046  | -0.054 |
| DS                | 32         | 2      | 0.063 | 0.058  | 0.406 | 0.152 | 0.160  | 0.056 | -0.05  | -0.050 | 0.000  | -0.155 | -0.042 | -0.113 |
| SS                | 15         | 3      | 0.257 | 0.142  | 1.733 | 0.242 | 0.242  | 0.002 | 0.003  | 0.009  | -0.005 | -0.062 | 0.02   | -0.081 |
| ZS                | 52         | 5      | 0.184 | 0.072  | 1.191 | 0.208 | 0.213  | 0.022 | -0.016 | -0.013 | -0.002 | -0.106 | -0.006 | -0.100 |
| NJ                | 30         | 5      | 0.253 | 0.104  | 1.733 | 0.242 | 0.242  | 0.010 | 0.003  | 0.007  | -0.004 | -0.085 | 0.02   | -0.105 |
| NR                | 32         | 4      | 0.181 | 0.09   | 1.219 | 0.207 | 0.211  | 0.018 | -0.017 | -0.014 | -0.003 | -0.111 | -0.004 | -0.106 |
| HK                | 30         | 3      | 0.131 | 0.082  | 0.867 | 0.183 | 0.189  | 0.029 | -0.031 | -0.029 | -0.002 | -0.127 | -0.021 | -0.106 |
| WZ                | 14         | 2      | 0.143 | 0.119  | 0.920 | 0.189 | 0.193  | 0.020 | -0.029 | -0.026 | -0.003 | -0.098 | -0.018 | -0.080 |
| XM                | 32         | 5      | 0.238 | 0.099  | 1.625 | 0.233 | 0.236  | 0.013 | -0.001 | 0.003  | -0.003 | -0.087 | 0.015  | -0.102 |
| SZ                | 14         | 2      | 0.264 | 0.136  | 1.000 | 0.245 | 0.250  | 0.021 | 0.008  | 0.012  | -0.002 | -0.062 | -0.015 | -0.047 |
| MZ                | 24         | 4      | 0.239 | 0.113  | 1.625 | 0.234 | 0.236  | 0.009 | -0.001 | 0.003  | -0.004 | -0.009 | 0.015  | -0.105 |
| PT                | 29         | 3      | 0.135 | 0.085  | 0.897 | 0.185 | 0.190  | 0.026 | -0.030 | -0.028 | -0.002 | -0.126 | -0.019 | -0.106 |
| QD                | 21         | 3      | 0.452 | 0.105  | 1.616 | 0.333 | 0.364  | 0.087 | 0.082  | 0.067  | 0.015  | -0.028 | 0.014  | -0.042 |
| NA                | 30         | 4      | 0.193 | 0.095  | 1.300 | 0.212 | 0.216  | 0.016 | -0.014 | -0.011 | -0.003 | -0.106 | 0.000  | -0.105 |

Population genetics and demography

Genetic differentiation among different populations was evaluated using the genetic distances of Tajima-Nei values (see Appendix S3). In general, pairwise distances were low ($-0.0387$ to $-0.18509$). The largest genetic differentiation occurred between QD and DS populations ($0.1359$, $P<0.001$). The pairwise distances were negative ($-0.0307$, $P=0.99$) for the two populations from the Yellow Sea (QD and DGD), indicating that the variation within populations was greater than the variation between populations. In the 10 populations from the East China Sea, the pairwise distances were also very low ($-0.01394$ to $-0.00514$) and insignificant ($P>0.05$), as was the case for the three populations from the South China Sea (SZ, HK and WZD, 0.0387 $\sim 0.1856$, $P>0.05$). The divergence of the populations calculated using CONTRIB showed that the absolute gene differentiation was largest for the most remote, isolated population, DGD ($DGst = 0.104$) followed by the other most isolated population, QD ($DGst = 0.104$, Table 2).

$F_{ST}$ values among the three groups (the Yellow, East China and South China Sea), were low (0.00613 to 0.18991, Table 3). The $F_{ST}$ value between the East China and South China Sea was 0.00613, whilst the $F_{ST}$ values between the Yellow Sea and the other two groups were relatively high (East China Sea, $F_{ST} = 0.18991$, $P<0.001$; South China Sea, $F_{ST} = 0.12820$, $P<0.001$). The absolute gene differentiation ($DGst$) calculated
using CONTRIB was highest in the YS group (0.115) as compared to the two other groups (ECS, 0.077; SCS, 0.070, Table 2). AMOVA analysis showed genetic differences among groups (FCT), genetic differences among populations within groups (FST) and genetic differences among populations (FST, Table 4), indicating the existence of relatively high genetic differences among all different group levels.

The Mantel test indicated a significant relationship (P = 0.046) between FST and geographic distance in all 15 populations, indicating evidence of isolation by distance, with geographic distance explaining 15% of the variation in genetic differentiation between populations of Cellana toreuma (r^2 = 0.15). However, such a relationship between genetic variation and geographic difference was not found among populations from the East China or South China Sea groups (P = 0.192), with only ~1% of the variation in genetic differentiation being explained by geographic distance (r^2 = 0.01, Fig. 3).

Tajima’s D and Fu’s Fs were used to test for neutrality, and the results in different locations were variable (Table 5). All Tajima’s D-values were negative. At the population level, populations from the Yellow Sea had insignificant negative values as did populations from the South China Sea (SZ, HK and WZD, Table 5). In contrast, all populations from the East China Sea showed

Table 3. Pairwise genetic distances between populations of Cellana toreuma separated into the Yellow Sea, East China Sea and South China Sea groups along the China coast1.

|            | YS group | ECS group | SCS group |
|------------|----------|-----------|-----------|
| YS group   | 0.18991  | 0.12820   | 0.000001  |
| ECS group  | <0.000001 | 0.00613   | 0.07207   |
| SCS group  | <0.000001 | 0.07207   | 0.00613   |

1The upper matrix shows the Tajima-Nei FST values and the lower matrix shows the P values.

Fig. 2. Haplotype frequencies of COI mtDNA. Haplotype frequencies of partial COI mtDNA from 15 populations of Cellana toreuma. In each circle, blue and red represent the proportions of H1 and H2, respectively. Inset: Neighbour-joining tree of partial COI mtDNA of the three groups (see Table 1 for population code).

doi:10.1371/journal.pone.0036178.g002
significant negative values indicating significant population expansion of *C. toreuma* in the East China Sea (Table 5). Mismatch distributions, to explore the distribution of the number of pairwise differences between haplotypes, were unimodal, matching the sudden expansion model (Fig. 4).

**Discussion**

A dominate haplotype (H1) was found to be ubiquitously distributed in all the studied populations of *Cellana toreuma* along the China coast. The high percentages and wide distribution of H1 in the 15 sampled populations indicates that a population expansion of this species has occurred. Evidence for the occurrence of a population expansion was supported by the significant negative values of the neutrality tests (Tajima’s D test and Fu’s Fs test), especially in the East China Sea populations. The unimodal mismatch distribution was also in accordance with an expected sudden population expansion [66]. These results, therefore, support the hypothesis that *C. toreuma* populations along the China coast had experienced a large-scale population expansion.

The population expansions and contractions were closely associated with Pleistocene-era environmental fluctuations [6]. The glacial and inter-glacial exchanges have had significant impacts on current phylogeographic patterns [18]. During the last glacial maximum (12,000 and 75,000 years ago), sea level decreased dramatically in the marginal seas of China (100–120 m in the South China Sea, and 130–150 m in the East China Sea [45]). As a consequence of sea level dropping, the East China Sea was reduced to an elongated trough, the Okinawa Trough, while the South China Sea became a semi-enclosed gulf [45]. Due to the southern shift of the polar front in the North Pacific, and the reorganization of the surface current systems, winter temperatures in the South China Sea were ~6–10°C colder than the present day, and seasonality was much stronger [45]. As the environmental conditions in the intertidal zone are affected by both marine and terrestrial factors, intertidal species experience stronger environmental stresses than animals living in the ocean [6]. The cold and dry conditions during the LGM would, therefore, be expected to eradicate most *Cellana toreuma* in northern China and can explain the post-LGM recolonization from southern to northern China after the LGM. The genetic connectivity of *C. toreuma* along the China coast can, therefore, be explained by a demographic expansion which may have been mediated by transport of larvae via the Kuroshio Current, China Coastal Current and the Yellow Sea Warm Current (see Fig. 1).

The relative higher nucleotide and gene diversity in the Yellow Sea group indicates that there are other important factors affecting the phylogeographic pattern of *Cellana toreuma* along the China coast. As described in Hewitt [67], low genetic diversity at relatively high latitudes, with a small number of alleles or haplotypes dominating disproportionately large areas, is a pattern most consistent with the hypothesis of a recent range extension from a southern refuge. In the present study, however, the nucleotide diversity and gene diversity in QD and DGD were higher than those of the southern populations, and haplotype No. 2 (H2) was common in the Yellow Sea group, but could not be found in populations from the East China or South China Seas. The pairwise genetic distance between the Yellow Sea group and the other two groups were relatively high, and AMOVA analysis suggested the presence of geographic structure in the populations. The Yellow Sea group was, therefore, relatively isolated from the other two groups. Three principal factors may potentially contribute to the observed geographic structure of *C. toreuma*

Table 4. Analysis of molecular variation for samples of *Cellana toreuma* along the China coast to investigate genetic differences in different levels.

| Source of variation     | d. f. | Sum of squares | Variance components | Percentage of Variation | Fixation Index | P value |
|-------------------------|-------|----------------|---------------------|------------------------|----------------|---------|
| Among Groups            | 2     | 2.753          | 0.01439             | 10.90                  | 0.10901        | 0.00000 |
| Among populations within groups | 12    | 1.263          | −0.00046            | −0.35                  | −0.00391       | 0.51026 |
| Within populations      | 403   | 47.53          | 0.11809             | 89.45                  | 0.10553        | 0.00000 |
| Total                   | 417   | 51.604         | 0.13202             |                        |                |         |

*doi:10.1371/journal.pone.0036178.t004*

Figure 3. Relationship between genetic distance and geographic distance. (A) Relationship between genetic distance and geographic distance for all populations; (B) Relationship between genetic distance and geographic distance for populations from the East China Sea and South China Sea groups.

*doi:10.1371/journal.pone.0036178.g003*
populations along the China coast: firstly populations survived in northern refuges during the LGM or, secondly, the existence of large areas of unsuitable habitat can form barriers that potentially isolated the Yellow Sea populations, or finally, that the observed genetic isolation is due to the effect of contemporary ocean currents and/or Yangtze River discharge.

There was only one mutational step between H1 and H2, and the genetic distance between the two haplotypes was very low, indicating the isolation of H2 was a relatively contemporary event. In southern Australia, a clear phylogeographic break in the distribution of the barnacle, *Catomerus polymerus*, was found due to the large expanse of sandy shores devoid of suitable rocky substrate for *C. polymerus* to settle on along Ninety Mile Beach [68]. The coastline between Qingdao and the Yangtze River is also composed of long stretches of unsuitable habitat for *C. toreuma* (400 km), such as salt marshes, which might act as possible barriers restricting the dispersal of *C. toreuma* larvae. In this region, however, there are intermittent islands, dams and harbours, which may provide suitable hard substrates for colonization by *C. toreuma* and act as stepping-stones for colonization, as has been described for rocky intertidal invertebrates in south-eastern Australia [69]. It seems unlikely, therefore, that there is a significant barrier caused by a lack of suitable substrata which is isolating the Yellow Sea populations, although this requires further confirmation.

The relatively higher nucleotide and gene diversities in the Yellow Sea group are, therefore, most likely related to population disjunctions, due to the impact of ocean currents and/or Yangtze River discharge. This explanation seems plausible as pelagic larval dispersal will be the major source of contemporary gene flow and hence distribution of *C. toreuma*. Reproduction in *C. toreuma* usually occurs in summer. In north Zhejiang, China, *C. toreuma* reproduces from June to September [70] and in Tanabe Bay, Japan, it breeds twice in April to June and September to October [71]. Although the exact length of the pelagic larval stage is unknown for this species, it varies between 4–18 days in congenerics [38]. In spring and summer, the plume of water from the Yangtze River discharge shifts in a northerly direction in parallel with the Taiwan warm current with a clockwise deflection (Fig. 1). The size and distance of deflection of the dilute water in summer is greater than in spring. As a result, the East China Sea coastal current, which flows from north to south along the coast in winter, is deflected at the mouth of the Yangtze River due to the increased river discharge in spring and summer [72]. The dilute plume of Yangtze River waters in spring and summer can also cause

**Figure 4. Observed pairwise differences and expected mismatch distribution.** Observed pairwise differences (bars) and the expected mismatch distributions (line) under the sudden expansion model of COI gene. (A) in all samples, (B) in Yellow Sea group, (C) in East China Sea group and (D) in South China Sea group. doi:10.1371/journal.pone.0036178.g004
a decrease in the salinity of the upper layer of the Kuroshio Current [73], which will affect nutrient concentrations [74], and subsequently phytoplankton biomass [75]. The disruption of the southward East China Sea coastal current due to the impact of the summer Yangtze discharge [76]. As S. horneri mature twice a year (in spring and autumn), the southward China Coast Current in winter can transport their propagules from the Yellow sea to East China Sea, and then to the South China Sea, and will not be affected by the barrier created by the summer Yangtze discharge. In contrast to S. horneri, the pelagic larval dispersal stage of C. toreuma is limited to only 4–18 days. Most importantly, the reproductive season of C. toreuma occurs only during summer, when the southward China Coast Current is interrupted by freshwater discharge from the Yangtze River which decreases salinity significantly, and therefore acts as a temporary barrier to the demographic expansion for northern C. toreuma populations. The phylogeographic patterns of intertidal species along the China coast are, therefore, closely related to species-specific life cycles and related ecological characteristics.

In general, therefore, both historic events and contemporary oceanographic conditions play important roles in the demographic expansion of the intertidal limpet, Cellana toreuma. Along the coast of China the interplay between these factors is strongly influenced by the timing of intense discharge from the Yangtze River. The coincidence of increased Yangtze River discharge and the resulting weakening of the China Coast Current in summer, when larvae of C. toreuma will be in the water column, act as a physical barrier, inhibiting the southern dispersal of limpet larvae and playing an important role in the phylogeographic patterns of this limpet along the China coast.

**Supporting Information**

**Appendix S1** The haplotypes frequencies of COI in different Cellana toreuma populations in China coast.

**Appendix S2** Neighbour-joining tree based on COI dataset with bootstrap values of 1,000 above each branch of interest with other Cellana limpets as outgroups.

**Appendix S3** Genetic pairwise distances of the COI mitochondrial gene between 15 Cellana toreuma populations.

**Acknowledgments**

The authors would like to thanks Prof. Xiaoqi Zeng (Ocean University of China) for providing samples in DGD; Prof. KH Chu and Drs. LM Tsang (Chinese University, Hong Kong), BKK Chan (Academia Sinica, Taiwan) and SJ Hawkins (University of Southampton, UK) for helpful and informative discussions and information and especially Dr. SJ Goldstien and anonymous reviewers for their constructive comments.

**Author Contributions**

Conceived and designed the experiments: Y-wD GAW TN C-hK. Performed the experiments: Y-wD GAW G-DH XZ. Analyzed the data: H-W Y-wD. Contributed reagents/materials/analysis tools: GAW Y-wD H-W. Wrote the paper: Y-wD GAW TN C-hK.

**References**

1. Carlton JT, Hodder J (1995) Biogeography and dispersal of coastal marine organisms: experimental studies on a replica of a 16th-century sailing vessel. Marine Biology 121: 721–730.
2. Bouchet P (2006) The magnitude of marine biodiversity. In: Duarte CM, ed. The exploration of marine biodiversity: scientific and technological challenges. Bilbao: Fundacio´n BBVA. pp 32–62.
3. Sagarin RD, Gaines SD (2002) Geographical abundance distributions of coastal invertebrates: using one-dimensional ranges to test biogeographic hypotheses. Journal of Biogeography 29: 985–997.
4. Avise JC (2000) Phylogeography: the history and formation of species. Cambridge, MA: Harvard University Press.
5. Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2000) Biogeography: A marine Wallace’s line? Nature 406: 692–693.

**Table 5. Tajima’s D and Fu’s Fs, and corresponding P values, and mismatch distribution parameters for 15 populations of Cellana toreuma and their associated groups along the China coast (see Table 1 for site and group abbreviations).**

| ID | Tajima’s D | Fu’s Fs | Mismatch distribution |
|----|------------|---------|-----------------------|
|    | D          | P       | Fs                    | P          | r (D) | r (Fs) | r (D) |
| QD | 0.33029    | 0.31900 | -0.27034              | 0.28100    | 0.60938 | 0     | 99999.000000 |
| DGD| -1.11781   | 0.01560 | -2.31340              | 0.04300    | 0.71875 | 0     | 99999.000000 |
| S  | -1.5945    | 0.17200 | -1.54636              | 0.02000    | 3.00000 | 0     | 0.36533   |
| ZS | -1.76473   | 0.01300 | -4.92636              | 0.00000    | 3.00000 | 0     | 0.23760   |
| NJ | -2.00763   | 0.00200 | -3.14992              | 0.00200    | 3.00000 | 0     | 0.32139   |
| PT | -1.50906   | 0.01500 | -2.31203              | 0.00600    | 3.00000 | 0     | 0.16396   |
| NRD| -1.32954   | 0.01400 | -3.48995              | 0.00000    | 3.00000 | 0     | 0.23271   |
| MZD| -1.51469   | 0.01500 | -3.02081              | 0.00100    | 3.00000 | 0     | 0.33115   |
| CW | -1.72954   | 0.01400 | -3.48995              | 0.00100    | 3.00000 | 0     | 0.23271   |
| XM | -1.88876   | 0.01000 | -4.54522              | 0.00000    | 3.00000 | 0     | 0.23871   |
| DS | -1.14244   | 0.12000 | -1.26483              | 0.05300    | 3.00000 | 0     | 0.06855   |
| NA | -1.73178   | 0.01300 | -3.38072              | 0.00000    | 3.00000 | 0     | 0.25225   |
| S  | -0.34144   | 0.20100 | 0.18574               | 0.30400    | 0.33008 | 0     | 99999.000000 |
| HK | -1.26161   | 0.06800 | -1.70822              | 0.01400    | 3.00000 | 0     | 0.24004   |
| WZD| -1.48074   | 0.07300 | 0.29648               | 0.34900    | 3.00000 | 0     | 0.10537   |

| Group level | Group | Total |
|-------------|-------|-------|
| YS          | 0.93178 | 0.0830 | -1.86278 | 0.07700 | 0.66016 | 0 | 99999.000000 |
| ECS         | -2.48839 | 0.00000 | -∞ | 0.00000 | 3.00000 | 0 | 0.23271 |
| SCS         | -1.83152 | 0.00400 | -∞ | 0.00000 | 3.00000 | 0 | 0.24004 |

| Total       |       | 0.672  | 0     | 99999.000000 |

doi:10.1371/journal.pone.0036178.t005
6. Markos PB, Hoffman JM, Emme SA, McGovern TM, Kreeger CC, et al. (2010) The ‘Expansion–Contraction’ model of Pleistocene biogeography: rocky shores suffer a sea change. Molecular Ecology 19: 146–169.

7. Tsang LM, Chan BKK, Wu TH, Ng WC, Chanterje T, et al. (2000) Population differentiation in the barnacle Chthamalus tenuispicu and recent connectivity across the Pacific and Indian Oceans. Marine Ecology Progress Series 364: 107–118.

8. Scheltema RS (1968) Dispersal of Larvae by Equatorial Ocean Currents and its effect on the Zoogeography of shallow-water Tropical Species. Nature 217: 1159–1162.

9. Scheltema RS (1971) Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. Biologia Marítima 10: 319–322.

10. Kyle CJ, Beaudoin EG (2000) Comparative population genetic structure of marine gastropods (Littorina spp.) with and without pelagic larval dispersal. Marine Biology 137: 835–845.

11. Cowen RK, Sponaugle S (2008) Larval Dispersal and Marine Population Connectivity. Annual Review of Marine Science 1: 443–466.

12. Connolly SR, Menge BA, Roughgarden J (2001) A Latitudinal gradient in intertidal community structure: evidence for an oceanographically based synthesis of marine community theory. The American Naturalist 151: 4: 311.

13. Connolly SR, Menge BA, Roughgarden J (2001) A Latitudinal gradient in recruitment of intertidal invertebrates in the northeast pacific ocean. Ecology 82: 1799–1813.

14. Marks PB (2004) “What’s larval got to do with it?” Disparate patterns of post-glacial population connectivity in two benthic marine gastropods with identical dispersal potential. Molecular Ecology 13: 597–611.

15. Southward AJ, Hawkins SJ, Burrows MT (1995) Seventy years’ observations of changes in distribution and abundance of zooplankton and intertidal organisms in an eastern English Channel in relation to rising sea temperature. Journal of Thermal Biology 20: 127–155.

16. Sagarin RD, Barry JP, Gilman SE, Baxter CH (1999) Climate related changes in an intertidal community over short and long time scales. Ecological Monographs 69: 49–665.

17. Rivadeneira MM, Fernández M (2005) Shifts in southern endpoints of geographic barrier? Molecular Ecology 18: 1887–1903.

18. Hewitt GM (2004) Genetic consequences of climatic oscillations in the Thermal Biology 20: 127–155.

19. Connell JH (1985) The consequences of variation in initial settlement vs. post-settlement connectivity of marine and stream benthic invertebrates. Trends in Ecology & Evolution 11: 322–326.

20. Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DD (2000) Connectivity of Nearshore Populations: Open or Closed? Science 287: 857–859.

21. Ayre DJ, Minchinton TE, Perrin C (2009) Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? Molecular Ecology 18: 1807–1903.

22. Nakano T, Sasaki T, Kase T (2010) Color polymorphism and historical biogeography of the Japanese patellagastropod limpet Cellana nigrolineata (Reeve) (Gastropoda: Patellidae). Zoological Science, 27: 811-820.

23. Connell JH (1972) Community interactions on marine rocky intertidal shores. Ecology 53: 359–373.

24. Branch GM (1981) The biology of limpets: physical factors, energy flow, and ecological interactions. Oceanography and Marine Biology Annual Review 19: 235–380.

25. Coleman R, Underwood A, Benedetti-Cecchi L, Aberg P, Arenas F, et al. (2006) A continental scale evaluation of the role of limpet grazing on rocky shores. Oecologia 147: 556–564.

26. Bier CE, Holland BS, Bowen BW, Toonen RJ (2007) Contrasting phylogeography in three endemic Hawaiian limpets (Cellana spp.) with similar life histories. Molecular Ecology 16: 3173–3186.

27. Coe WR (1953) Resurgent populations of littoral marine invertebrates and their dependence on ocean currents and tidal currents. Ecology 34: 225–229.

28. Nakano T, Ozawa T (2004) Phylogeny and historical biogeography of the Japanese patellogastropod limpet Cellana nigrolineata (Reeve) (Gastropoda: Cellana nigrolineata (Reeve)). (Phaeophyceae, Heterokontophyta) in northwestern Pacific: a top cetacean predator: the harbour porpoises in European water. Molecular Ecology 35: 3259–3268.

29. Nakano T, Sasaki T, Kase T (2010) Coloration and historical biogeography of the genus Patella based on mitochondrial DNA sequence data. Journal of Experimental Marine Biology and Ecology 325: 95–110.

30. Southward AJ, Hawkins SJ, Burrows MT (1995) Seventy years’ observations of changes in distribution and abundance of zooplankton and intertidal organisms in an eastern English Channel in relation to rising sea temperature. Journal of Thermal Biology 20: 127–155.

31. Sagarin RD, Barry JP, Gilman SE, Baxter CH (1999) Climate related changes in an intertidal community over short and long time scales. Ecological Monographs 69: 49–665.

32. Ayre DJ, Minchinton TE, Perrin C (2009) Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? Molecular Ecology 18: 1807–1903.
62. Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123: 585–595.
63. Fu YX (1996) New statistical tests of neutrality for DNA samples from a population. Genetics 143: 557–570.
64. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution.
65. Bohonak AJ (2002) IBD (Isolation by Distance): A Program for Analyses of Isolation by Distance. Journal of Heredity 93: 153–154.
66. Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9: 552–569.
67. Hewitt GM (1999) Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68: 87–112.
68. York KL, Blacket MJ, Appleton BR (2008) Bassian Isthmus and the major ocean currents of southeast Australia influence the phylogeography and population structure of a southern Australian intertidal barnacle *Catanomus polymerus* (Darwin). Molecular Ecology 17: 1948–1961.
69. Hidas E, Cost, TL, Ayre DJ, Minchinton TE (2007) Is the species composition of rocky intertidal invertebrates across a biogeographic barrier in south-eastern Australia related to their potential for dispersal? Marine and Freshwater Research 58, 835–842.
70. Wang Z, Wu C (2000) Study on the age and growth of *Cellana trauma* in north Zhejiang coastal area. Journal of Zhejiang Ocean University 19: 316–326.
71. Ohgaki S (2005) Ecology of molluscs around Tanabe Bay, a review. Argonauta (Newslet Kansai Mar Biol Sem Ser) 11: 27–46.
72. Liu XQ, Yin BS, Hou YJ (2008) The dynamic of circulation and temperature-salinity structure in the Changjiang mouth an dits adjacent marine area. Oceanologia et limnologia Sinica 39: 312–320.
73. Wu ZY, Chen HX, Liu N (2010) Relationship between east China Sea Kuroshio and climatic elements in East China. Marine Science Bulletin 12: 1–9.
74. Jiang CB, Zhang LJ, Wang F (2006) A study of pCO2 in the surface water of the southern Yellow Sea in summer II: The respective contribution of the upwelling and the Yangtze River diluted water to the Air-Sea of CO2 flux. Periodical of Ocean University of China 36: 147–152.
75. Liu J, Zhu JR, Zhang J, Wu H, Luo WJ (2011) Distribution of phytoplankton biomass and its relationships with the environmental factors off the Changjiang (Yangtze River) Estuary. Journal of Fisheries of China 35: 74–87.
76. Hu Z-M, Uwai S, Yu S-H, Komatsu T, Aisaka T, et al. (2011) Phylogeographic heterogeneity of the brown macroalga *Sargassum horneri* (Fucaceae) in the northwestern Pacific in relation to late Pleistocene glaciation and tectonic configurations. Molecular Ecology 20: 3894–3909.