

A molecular clock for Arctic marine invertebrates

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Abstract

In the Arctic region, the opening/reclosure events of the Bering Strait provide a calibration point to estimate molecular divergence rates in northern marine taxa. Here, we used the novel “iterative calibration” approach to incorporate the complete glacial history of the Bering Strait for clock dating. Using publicly available sequences of the cytochrome c oxidase subunit I (COI) gene, we explored patterns of molecular divergence across 91 trans-Bering sister clades in echinoderms, molluscs, polychaetes, and arthropods. This is the first large-scale study for clock calibration in Northern marine invertebrates. The results presented here will advance our ability to date evolutionary events in the marine realm and will expand our understanding of the impacts of prior climatic changes upon the history of life.

Introduction

Divergence times for Arctic marine lineages have commonly been estimated based on calibrations from geographically distant taxa. However, due to evidence of rate heterogeneity among taxa and environments, it is essential to pursue clock calibrations for Northern lineages. The opening and re-closure events of the Bering Strait provide an exceptional resource for calibrating the molecular clock in Northern marine taxa. This source of calibration has been barely explored in the past^{1,2}. The Bering Strait first opened 5.4–5.5 Ma³. However, the major marine migration from the Pacific to the Arctic Ocean took place 3.5 Ma^{4,5}. The migratory events were followed by a maximum glacial (2.4 to 3 Ma) episode, with a decline in the sea level and the consequent closure of the Bering Strait⁶. Since then, successive episodes of formation and retreat of glaciers occurred, shaping the distribution of Northern marine fauna. In this study we explored divergence patterns of 91 trans-Bering sister pairs of lineages inhabiting the Pacific vs. Arctic-Atlantic Oceans using the iterative calibration approach and the Bering Strait glacial history to calibrate the molecular clock.

Methods

1. Preliminary identification of potential trans-Bering sister clades using neighbour-joining trees in BOLD⁸.
2. Publicly available COI-5P sequences downloaded from BOLD and aligned in MEGA v7⁹.
3. Confirmation of trans-Bering sisters using maximum-likelihood trees.
4. Tajima’s relative rate test¹⁰.
5. Saturation test.
6. Kimura two-parameter (K2P) distances.
7. **Iterative calibration approach**¹¹ to find the best calibration date according to the glacial history of the Bering Strait.
 - Assigning a starting calibration date to a selected reference node and then estimating divergence times for the other sister clades.
 - Compare with the geological time scale.
 - If necessary, re-assign ages to sister clades and compare with the geological history.
 - Following the process until finding concordance between genetic divergences and the geological history of the Bering Strait.

Results and Discussion

Divergences between trans-Bering sisters ranged from 0.12% to 26.37% K2P. Assuming simultaneous isolation of all sister pairs during the major trans-Arctic interchange (3.5 Ma), as commonly assumed in the literature, would imply high variability in evolutionary rates. However, rate heterogeneity was not the major explanation since the molecular clock hypothesis was rejected for only five pairs, and whole-tree analyses for select taxa indicated only modest clock deviations. Thus, the results strongly support previous research suggesting multiple pulses of trans-Bering migrations². Our results suggest a rate of K2P divergence of 2.8%/MY in echinoderms, 3.2%/MY in molluscs, 5–5.2%/MY in arthropods, and 3.5–4.7%/MY in polychaetes. These rates contrast with a highly-cited low divergence rate reported for tropical alpheid shrimps (1.4%/MY)¹². The prevalent use of the alpheid rate could have led to a systematic underestimation of rates for dating marine phylogenies.

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Table 1 Summary of molecular clock calibrations for the mitochondrial cytochrome c oxidase subunit I gene (COI) in the four groups of Northern marine invertebrates used in this study.

Taxon	Total number of sister pairs	Range of divergences among sister pairs (% K2P)	Divergence rate estimate (% K2P per MY)
Echinodermata	16	0.45–15.45%	2.8%
Mollusca	26	1.57–21.87%	3.2%
Arthropoda	29	0.91–26.37%	5.0–5.2%
Polychaeta	20	0.12–15.97%	3.5–4.7%

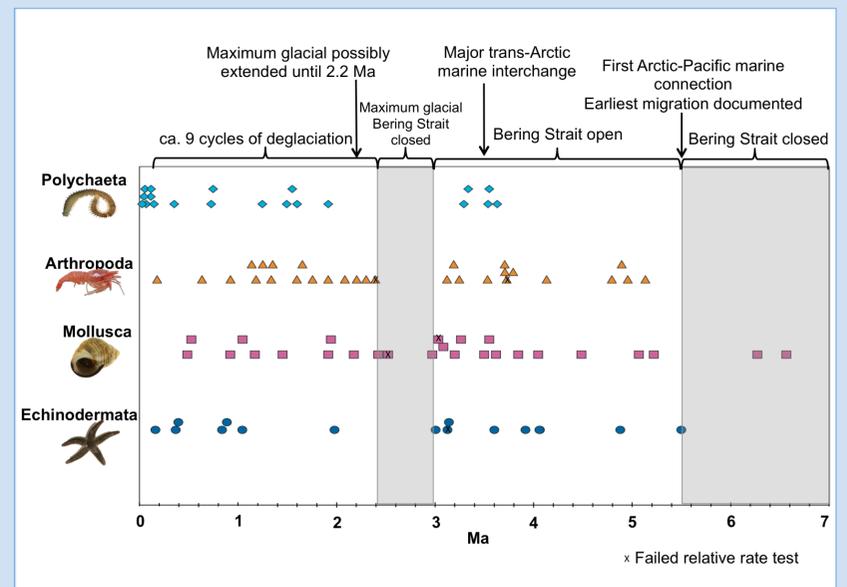


Figure 1. Divergence time estimates for Northern marine echinoderms, molluscs, arthropods, and polychaetes using K2P divergences and the best calibration according to the iterative calibration approach. The shaded boxes show the two periods of time when trans-Bering migrations were extremely unlikely, during the maximum glacial (2.4–3 Ma) and before the earliest well-supported opening of the Bering Strait at 5.5 Ma.

Conclusions

This study provides strong evidence of multiple pulses of trans-Bering migrations in all four groups of marine invertebrates. The iterative calibration was successful for calibrating the molecular clock for Northern marine taxa using the Bering Strait despite the complex glacial history. By integrating genetic, biogeographic, and fossil evidence, and by using a substantial number of sister clades, we anticipate more accurate calibrations than when using simplistic assumptions. The new rates of molecular evolution presented here will advance our ability to date recent evolutionary events in the marine realm. However, more studies are still needed to further understand evolutionary rates in marine taxa and to expand our understanding of the impacts of prior climatic changes upon the history of life.

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