Cytochrome oxidase I DNA sequences from Pollicipes elegans collected at the University of Hawaii in 2015 (Gene flow across the tropics project)

Website: https://www.bco-dmo.org/dataset/629208
Data Type: experimental
Version: 2015-12-17

Project
» Gene Flow and Divergence Across the Equatorial Tropical Marine Barrier: Past, Present and Future (Gene flow across the tropics)

Contributors	Affiliation	Role
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Dataset Description

This dataset was published in Marchant et al (2015).

Samples were collected along the Pacific coasts of Mexico, El Salvador and Peru.

Related Datasets:
Microsatellite primers for Pollicipes elegans
Pollicipes elegans aggregation genotypes
Pollicipes elegans larval genotypes

Related References:

Marchant, S., Moran, A. L., Marko, P. B. 2015. Out of the tropics or trans-tropical dispersal? The origins of the disjunct distribution of the gooseneck barnacle Pollicipes elegans. Frontiers in Zoology 12: 39. doi: 10.1186/s12983-015-0131-z

Methods & Sampling

In the Acquisition and Processing sections, [#] refers to the references cited in the publication Plough et al. BMC Evolutionary Biology, 2014.

Sampling and study population

Barnacles were collected from one 20-m-long rocky outcrop, approximately near Punta Gaspareno, Baja California, Mexico (23°10'58.09 oN, 110° 8'26.51 oW) in October 2011. Aggregations of barnacles at seven different densities--1, 2, 4, 12, 17, 22, and 44 individuals per 10 cm2-- were sampled haphazardly using a 10 cm2 quadrat (see Table 1). We found few individuals brooding embryos in the lowest density groups; only one of eight groups with a density of two individuals per10 cm2 and none of the four solitary individuals had embryos. Lower densities (1 or 2 individuals) were sampled more than once in an attempt to find individuals that were brooding embryos for paternity analysis. In total, 119 barnacles were sampled, but of these only 21 individuals exhibited brooding embryos, 14 of which yielded larvae for paternity analyses (Table 1; larval culturing description below). Individual barnacles were scraped off of rocks with 1 mm-thick metal paint scrapers and care was taken minimize damage to the bottom of the peduncle so that adults were brought back alive. Animals from each aggregation were then placed in individual zip-lock bags and kept moist with rinses of fresh seawater every 6-8 hours until they arrived at the laboratory ~24 hours later.

Larval culturing and tissue sampling
Prior to extraction of the disc-shaped embryo sacs (lamellae), each individual's peduncle was dipped in a 10% bleach solution, the capitulum was rinsed in 90% ethanol, and then the whole animal was rinsed thoroughly with 35 ppt artificial seawater (ASW, Instant Ocean). Lamellae (both discs) were extracted from the mantle cavity with forceps, rinsed with ASW, and transferred to clean, 100 ul plastic beakers containing 50 ul of fresh ASW treated with 1 mg/l each of streptomycin and penicillin to limit bacterial and fungal growth [71]. Larvae from each brood were reared in separate cultures in the dark at 25°C [71]; each culture was checked daily for hatching. After hatching, swimming larvae were transferred to new beakers and fed with Rhodomonas salinas and Isochrysis galbana at concentrations of 10,000 cells ml-1 each for ~48 h until sufficient numbers of stage-II larvae could be collected (50 or more) and preserved in 70% ethanol. Larvae were fed for 48 h after hatching because larger larvae yielded more DNA, facilitating individual genotyping. Peduncle tissue of mothers with broods that had 50 or more stage-II larvae were sampled and preserved in ethanol.

DNA was extracted from ~25 mg of adult muscle tissue using a modified CTAB protocol (Doyle and Doyle 1987) with two chloroform/isoamyl isolations and two 70% EtOH washes. Precipitated adult DNA was re-suspended with Qiagen EB buffer (10 mM Tris, ph 8.5; Qiagen Valencia CA). DNA from larvae were extracted individually in 200ul 96-well PCR plates using 25 ul of extraction buffer comprised of 0.5% tween, TE buffer (10 mm Tris, 1 mM EDTA), and 2.5 ul 20 mg/ml Proteinase k (Bioline). Larval extractions were incubated at 60°C for 4 hours followed by 30 minutes at 95°C and stored at -20°C. Raw (unprecipitated) larval extractions were used directly in PCR.

Genotyping and paternity analysis
Brooding individuals (mothers) and offspring were genotyped with at least three of five loci: Pole 1, Pole 8, Pole 25, Pole 29, and Pole 44 [72]. These loci have a high number of alleles (5-23) and gene diversity (average expected heterozgosity=0.59), show no evidence of null alleles, and exclusion probabilities calculated from the four most commonly genotyped markers (Pole 1, Pole 8, Pole 25, Pole 44) showed high discriminatory power (0.94; genotype data from Gaspareno, Mexico in [72]). PCR was carried out as described in Plough and Marko [72] and fragment analysis was run on the ABI 3100 sequencer at the Arizona State University DNA Lab. Electropherograms were scored by eye using LIZ600 (Applied Biosystems) as an internal size standard on the Peak Scanner software v. 1.0 (Applied Biosystems). Note that because P. elegans is hermaphroditic, all individuals, including brooding mothers are potential fathers.

Paternity analysis was performed using the program GERUD 2.0 [73]. The software does not allow for missing data, so only larvae that successfully amplified at all loci were included in the analysis of each brood. In one brood (c22), two larvae (0.48% of all genotyped larvae) were each homozygous at a single microsatellite locus for an allele that differed from the mother's, violating assumptions of Mendelian segregation. These larvae were removed from the analysis as possible contaminants from another brood but de-novo mutation at this locus could also explain the observed segregation pattern (e.g. [74,75]). Multiple genotype array solutions for the fathers were ranked by likelihood using the default test for Mendelian segregation. Though GERUD2.0 has the option of ranking solutions by allele frequencies from a reference or base population, we were not able to use this feature because some of the broods exhibited rare alleles not present in the genotype data from [72]. To determine if offspring were likely sired by fathers in the same physical aggregations, we examined whether the alleles observed in larval broods were also present in the genotypes of adults from the same aggregations in which those broods were collected. We compared larval and adult genotypes from each of two low density aggregations (2 individuals/10 cm2 and 4 individuals/10 cm2) and eight broods from three high density aggregations (17, 22, and 44 individuals/10 cm2; Table 1). All of the adults within the two low-density groups were genotyped, but in the three higher density aggregations we were able to genotype only ~80-95% of the adults because some individuals were damaged during collection and/or transport, compromising the quality of the DNA.

Power analysis of paternity
To determine the power to detect different levels of multiple paternity given the offspring sample size and the number of contributing fathers, we ran simulations using GERUDSIM 2.0. We determined the proportion of simulations out of 1000 that correctly assigned the true number of fathers (from 2-5), given various sample sizes of larvae genotyped (range, two - 96; actual mean sample size across the 14 broods =29.74) and the population allele frequencies of the four most commonly used markers (Pole 1, Pole 8, Pole 25, and Pole 44) from Baja California, Mexico [72]. Simulations were run assuming that the mothers' genotype was known and that the total offspring number per female (the average number of fertilized eggs) was 5000. In the simulations, five thousand offspring is then split among the true number of fathers for a given simulation scenario (e.g. for the 3 father simulation with equal reproductive contribution, each would be assigned 1666 offspring). Fecundity in P. elegans has not been measured systematically, but appears to range from a few thousand to 10's of thousands of eggs per female based on observations from this study, and estimates for the related barnacle Pollicipes pollicipes are similar [76]. Simulations with greater than 5000 total offspring ran extremely slowly in GerudSim2.0, but a few trial runs with 25,000 vs 5,000 total offspring yielded similar power results, so we set the total number of offspring in a brood to 5000. We also determined the power to detect the true number of fathers in a brood when there was skew in paternal contribution: 1/5 the contribution from one father and equal contributions from the others. These simulations were run only at the approximate mean offspring sample size (30). For example, in the case of five fathers, we assigned one father only 200 offspring and the other fathers 1,000.

Data Processing Description

Statistical and genetic analyses
Linear regression analysis of the effect of density on paternity was performed in the R statistical software package, v. 2.11.1 [77]. To examine skew in the reproductive contribution of putative fathers, Chi-square goodness-of-fit tests were run in R 2.11.1, with the null hypothesis of equal reproductive contribution. We also examined the association between relatedness of mates (calculated from the reconstructed paternal genotypes and the observed genotypes from the mother of each brood) and proportional paternity success. Relatedness was estimated with the Lynch & Li method [78] in COANCESTRY[79], because it performs well in a number of situations (e.g. [80]). Given the structure of the data (proportions within each brood sum to 1 and are grouped by female) we used a non-parametric, permutation-based approach to assess the significance of the relationship between relatedness and proportional siring success, because it makes fewer assumptions than analysis of variance or linear mixed-model methods. We implemented a permutation-based test of correlation using Spearman's Rho (a non-parametric analog to Pearson's correlation coefficient) that shuffles relatedness values while holding proportion sired static, within each female. The correlation is estimated for the true data, and then calculated after each permutation, and the number of permutations (out of 10,000) in which the permuted value is greater than the actual value is tallied for the one-tailed statistical test. This analysis was performed in R 2.11.1 (see Additional file 2 in publiation).

Adults from three high density aggregations of barnacles (44, 17, and 22 individuals per 10 cm2) were genotyped to assess relatedness among adults and possible fine-scale population structure. The relatedness estimator of Li [78] with weighting by locus [81] was calculated in the program STORM[82]. STORM calculates relatedness within a group or population and uses a permutation procedure to shuffle individuals across populations, creating a distribution of expected relatedness values for each group and overall, against which significance can be assessed. We calculated relatedness in the three aggregations, performing 10,000 permutations to determine if barnacles in these aggregations showed greater relatedness than expected by chance. For these calculations, data from eight microsatellite markers were used (Additional file 1: Table S1). Allele counts, heterozygosities, and tests of Hardy Weinberg Equilibrium were calculated with ARLEQUIN v. 3.5 [83]. We also examined fine scale population structure of the three aggregations using principle coordinates analysis (PCoA) and standard F-statistics. PCoA analysis was performed with the GENALEX 6.2 software [84], and pairwise and overall Fst was estimated with GENETIX using 10,000 permutations [85].

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Data Files

File
MtCOI_accessions.csv (Comma Separated Values (.csv), 23.70 KB) MD5:82e65442d0ebad706936d5d5476f0d6d Primary data file for dataset ID 629208

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Related Publications

Marchant, S., Moran, A. L., & Marko, P. B. (2015). Out-of-the tropics or trans-tropical dispersal? The origins of the disjunct distribution of the gooseneck barnacle Pollicipes elegans. Frontiers in Zoology, 12(1). doi:10.1186/s12983-015-0131-z
Results

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Parameters

Parameter	Description	Units
species	species name	unitless
gene	sequence gene	unitless
location	location sample was collected	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
NCBI_accession	NCBI accession number	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	ABI 3100 sequencer at the Arizona State University DNA Lab
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

Marko_lab_2015

Website	https://www.bco-dmo.org/deployment/627824
Platform	Marko_lab
Start Date	2013-10-01
End Date	2015-05-31
Description	genetics lab study on barnacle paternity

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Project Information

Gene Flow and Divergence Across the Equatorial Tropical Marine Barrier: Past, Present and Future (Gene flow across the tropics)

Coverage: Tropical eastern Pacific

Description from NSF award abstract:
The marine communities of the northern and southern hemispheres are kept distinct by warm water and strong currents near the equator. Nevertheless, many taxa have established "antitropical" distributions, occurring in temperate zones on either side of the tropics but not within the warmest tropical regions. Although paleontological studies and molecular phylogenies provide information about the timing and direction of trans-tropical colonization, the existence of single species with antitropical distributions provides an opportunity to begin to investigate several fundamental questions about the recent history of and future prospects for biological connectivity between the northern and southern hemispheres. This project focuses on characterizing connectivity across the tropics in the antitropical gooseneck barnacle *Pollicipes elegans*. This eastern Pacific species is found in both hemispheres but is absent from the warmest waters immediately to the north of the equator. Previously-collected data show that mtDNA haplotype lineages are shared between northern and southern hemisphere populations, and that the separation between north and south has been relatively recent, within the last 160,000 years. This research has two main goals. First, the investigators will use multi-locus sequence data and microsatellite loci to estimate the time of separation of northern and southern populations and how much gene flow has occurred since that split, both over evolutionary timescales and across recent generations. The second goal is to characterize larval thermal tolerance and performance as a means to test the hypothesis that temperature is a barrier to larval dispersal across the warmest tropical

regions in *P. elegans*. Although several other hypotheses can potentially explain what keeps *P. elegans* out of the tropics, such as the isolating effects of the equatorial boundary currents or post-settlement processes, assessing the temperature sensitivity of the dispersive larval stage will provide the first important physiological test of how temperature limits connectivity. Understanding the history of gene flow and the temperature tolerance of dispersive larvae will provide insight into the impacts of recent and projected increases in tropical sea surface temperatures on recent and future patterns of connectivity between the hemispheres.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1419986

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