

Chapter
20**Genetic and Genomic Approaches to
Understand Climate Mediated Adaptation
in Marine Organisms****Sandhya Sukumaran**

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Climate induced variations in natural populations

Populations of marine organisms respond to environmental and climatic fluctuations depending on the dynamics internal to each population, trophic interactions and several other extraneous factors. Due to the multitude of factors the relationship between an ecological or physiological responses and climatic fluctuations are often confounded. But the effects of climate change have become more important recently due to the rise in temperature levels in the oceans of the world and it is imperative to understand the relationship between climatic forcing and organismal responses. The first level of response to any extraneous factor is at cellular level or at the level of genome. Environment mediated selection plays a major role in bringing about adaptive evolution in response to a changing climate. The techniques in genetic and genomics can be used to understand climate mediated selection and evolution at individual, population and ecological levels.

Organismal evolution to changing climate takes place either by exploiting new resources in the new conditions or by tolerating the conditions to which it is not optimally adapted. Range expansions to temperate latitudes have been explained in many species of fishes in response to a changing climate and this takes place in order to utilize the novel resources. Another adaptive mechanism to utilise novel food resources is to extend the life cycle events or phenology which also has been recorded in many marine organisms.

Even though marine organisms can adapt to stressful conditions by range expansions, many sedentary organisms like mussels, clams and oysters have to face the effect of environmental stressors by tolerating conditions which are not optimal for their survival. The conditions may be extreme temperature levels,

ocean acidification, increased or decreased levels of salinity and many other factors. Such extreme conditions may select for altered spawning time or other phenological changes like flowering time in the case of plants. Organisms may change to altered food resource or their sex ratio may get affected due to temperature fluctuations.

The genetic composition of an organism can be affected by changes in allele frequencies for adapting to altered environmental conditions. If an allele provides tolerance capacity to increased temperature conditions in a population, the frequency of that allele will increase in populations over time as it provides improved fitness. But instead of a genetic change, phenotypic plasticity will also bring about better fitness to a particular environment. Phenotypic plasticity does not involve any change in the genetic constitution of an organism. Instead, same genotypes may exhibit different phenotypes to suit to that particular environment and varying gene expression patterns may play a role in bringing about phenotypic plasticity. The change in allele frequency can also bring about phenotypic plasticity which is regulated through complex networks and pathways.

Genetic and genomic methods for investigating climate mediated adaptive evolution

Genome scans, transcriptome profiling, association and mapping analysis in addition to candidate gene based approaches facilitate detection of loci important in adaptation to a changing climate. Several investigations have proved that adaptive evolution at genetic level is possible in genes which influence traits like body size, thermal responses, reproductive timing and dispersal. In addition, epigenetic changes which are heritable also will bring about climate mediated evolution.

Genome scan approaches can be used to undertake population level comparisons and detect differentiated gene regions as against expected from neutrality using large number of markers. Thus the effects of selection can be differentiated from population level processes. Clinal comparison of populations collected along clines will provide information regarding selection in response to geographic variations. But delineating the effects of climate change from other effects needs cautious approach.

Microarrays can be employed to hybridize DNA from highly diverged areas of the genome which may involve cline ends and this provide important information regarding parts of the genome involved in adaptive divergence. Candidate

genes linked to adaptive divergence can be studied along gradients based on known polymorphisms and effects on genes can be investigated through functional analysis.

Transcriptomic responses could be studied across gradients to understand differential gene expression patterns and to identify gene and gene networks that are differentiated between populations and cline ends. Mapping of quantitative trait locus can be carried out for strains which have been made homozygous for further comparison of quantitative traits among clines.

Epigenetic mechanisms have been presumed to play a major role in environment mediated gene expression and heritability of the acquired traits. Epigenetics refers to inherited changes in gene expression without variations in gene sequences. Several epigenetic mechanisms have been proposed like methylation of cytosine, modification of chromatin structure and changes in gene regulation induced by small RNAs. Phenotypic traits can be transferred from parents to offspring epigenetically without any change in gene sequences which is a very rapid form of adaptation. Stressful conditions lead to epigenetic alterations which mobilize transposable elements causing genetic modifications.

Next Generation sequencing methods for rapid detection of adaptive variation

Next generation sequencing methods refer to a variety of advanced techniques used to decipher the genetic code; Adenine, Thymine, Cytosine and Guanin. Massively parallel sequencing has been made possible by the advent of NGS techniques as compared to Sanger sequencing.

Second Generation Sequencing techniques

The Roche 454 platform

Roche454 platform/pyrosequencing was the first advanced next generation sequencing platform. DNA libraries can be constructed by any method which gives rise to a mixture of short, adaptor flanked fragments followed by emulsion PCR for clonal amplification of target sequences. Sequencing reactions are carried out using pyro-sequencing method. Sequencing by 454 produces reads of good read lengths and it can produce approximately 4,00,000 reads per instrument with lengths of 200-300bp. Homopolymers will increase the error rate and the most common error type is insertions and deletions.

Illumina Genome Analyzer

Illumina Genome Analyzer is the most popular advanced sequencing machine. Any method can be employed for production of libraries giving rise to a mixture of adapter-flanked fragments which are several hundred base pairs in length. A bridge PCR is used to amplify sequences. Many million clusters are amplified to specific locations within each of eight independent lanes on a single flow-cell and eight independent libraries can be sequenced in parallel at each run of the instrument. Read lengths of upto 35bp are possible currently with higher error rates at longer reads. Most common error type is substitutions.

AB SOLiD

In AB SOLiD, sequencing can be carried out by any method, which gives rise to a mix of short, adaptor flanked fragments. Using emulsion PCR clonal sequencing is carried out and amplicons captured to the surface of 1 μ m paramagnetic beads. Sequencing by synthesis is carried out using DNA ligase.

HeliScope

Clonal amplification is not required in HeliScope. Sequencing by synthesis is carried out in HeliScope with the help of a highly sensitive fluorescence detection system which carry out direct interrogation of single DNA molecule. Random fragmentation along with poly A-tailing is carried out for preparation of template libraries which are subsequently captured to surface bound poly-T oligomers by hybridization producing a disordered array of primed single molecular sequencing templates. Template dependent extension of surface-captured primer-template is facilitated by adding DNA polymerase and a single species of fluorescently labelled nucleotides at each cycle.

Third Generation Sequencing Technologies

Third generation sequencing technologies are characterised by novel chemistry, less operation time, desktop design and reduced operation cost. Pacific Biosciences real time single molecule sequencing (PacBioRS), Complete Genomics combined pre anchor hybridization and ligation (cPAL) and Ion Torrent of Life Technologies, Inc. are the major third generation sequencers. Third generation sequencing is also known as long-read sequencing which read nucleotides at single molecule level unlike second generation methods which require breaking of long fragments of DNA to small pieces and inferring nucleotide sequences by amplification and synthesis. PacBioRS is a real time single molecule- single polymerase sequencing platform capable of producing reads upto 1000bp. The chips comprise zero-mode wave guided (ZMW) nano

structures consisting of holes of size 100nm. Sequencing by synthesis is carried out in these holes by DNA polymerase enzyme with the help of phospholinked nucleotides which are labelled with fluorophores and introduced sequentially. The nucleotide incorporation kinetics can also be monitored using this instrument which helps to gather epigenetic information in the future.

Complete Genomics employ a combined approach of probe-anchor hybridization and ligation sequencing (cPAL) with the highest throughput among third generation sequencers. The method employs rolling circle amplification of small DNA sequences into the nanoballs form and further the sequence of nucleotides determined by ligation method. Using this approach, several DNA nanoballs could be sequenced per run with low costs. Ion Torrent technology is one of the most versatile and cheap methods and at present this equipment is supplied as a personal genomic machine (PGM). This benchtop instrument is being used widely by researchers and medical practitioners. The technology is based on proton release during nucleotide incorporation by DNA polymerase.

Fourth Generation Sequencing Technologies

Fourth generation sequencers like portable MinION machine, the benchtop GridION and high-throughput, high sample number PromethION offered by Oxford Nanopore systems are based on nanopore technology. These sequencers can sequence the entire genome of any organism rapidly and with very low cost. Nanopore based technologies originated based on the idea of coulter counter and ion channels. When an external voltage is passed through, particles which are smaller than pore size are passed through the pore and pores which are of nanometer size can be embedded in a biological membrane or in solid state film and consequently separating the reservoirs which contain conductive electrolytes into cis and trans compartments. When voltage is applied, electrolyte ions in solution will move through the pore electrophoretically generating ionic current signals. When negatively charged DNA molecule in the cis chamber blocks the pore, the current gets blocked interrupting the current signal. Then the physical and chemical properties of the target molecules can be calculated by statistical analysis of the amplitude and duration of current blockades which occurred transiently from translocation events.

Suggested Reading

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