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2.5 ToR e) Genomic approaches of adaptation of marine organisms in changing environments: what can populations tell us about genes underlying phenotypic changes and what can genes tell us about adaptive evolution of populations?

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Justification: Genomics of marine organisms can contribute to better understand how they can adapt to variation of environmental factors in the wild or under aquaculture conditions. In the wild, environmental variation can result from climate change, acidification of oceans, increasing levels of pollutants or fisheries. In aquaculture, adaptation can result from changes in rearing practices or to the extension of new pathogens. Adaptive responses can have phenotypic and genetic components that must be disentangled to model the evolutionary response of species.

Firstly, **genetically based phenotypic differences between wild or culture populations** have been demonstrated in many marine species. In these cases, **genome scans**, based on large numbers of genetic markers using high throughput genotyping technology, can identify regions of the genome associated with these differences and therefore resulting from response to differential selection pressures. When mapped on the genome, these markers contribute to identify QTLs and the genetic architecture of the concerned traits. Secondly, analysis of **sequence variation of coding and**

non-coding parts of the genome can be used to infer the role of selection on the shaping of the observed molecular diversity. Thirdly, **transcriptome sequencing,** revolutionized by the new generation of sequencing technologies, strongly facilitate the identification of genes differentially expressed in organisms exposed to different environmental conditions, or resulting from divergent selection in the wild or under aquaculture conditions. Candidate genes should then be validated using functional genomics approaches (i.e. reverse genetics, mutagenesis, RNAi...). They can be used for gene assisted selection or for population management purposes. Finally, both approaches (i.e. genome scans and transcriptome studies) can be **merged combined through eQTL** and genetical genomics studies, inferring genetic and environmental variance components associated with transcriptional abundances underlying adaptive traits. Such approaches provide further links between adaptation of marine organisms and the molecular bases of the concerned traits.

Novel genomics approaches aiming to better describe and understand these processes will be reviewed in the present ToR and study cases concerning fish and shellfish will be presented. Current developments will be described, highlighting the potentials and limitations of these approaches to contribute to better manage marine biodiversity.

Adaptation is a key component of sustainability in a changing environment. For living organisms, two main components must be distinguished: (1) phenotypic plasticity of an individual facing variable environmental conditions and (2) genetic polymorphism within a species allowing its potential adaptation to a given range of environments. Distinction between adaptive and non-adaptive evolution and elucidation of the genetic basis of adaptive population divergence is a goal of central importance in evolutionary biology. Genetically based polymorphism for traits involved in spatial or temporal adaptation can lead to differentiation over time or space if other evolutionary forces, such as gene flow, do not counterbalance the effect of local selection. Direct demonstration of the effect of selection – relative to other evolutionary forces - on local adaptation is one of the goals of evolutionary biology. Studies have long been – and still are – based on the analysis of phenotypic traits varying between populations in space or time. The increasing ability to obtain genomic information has opened novel possibilities to distinguish adaptation from other evolutionary forces by tracing its footprints at the molecular level. Establishment of functional links between the phenotypes and genotypes is also greatly facilitated by genomics and reverse genetics. Different approaches can be distinguished to demonstrate adaptive processes. They can be classified in two different groups:

- At the phenotypic level, the comparison of individuals sampled in different populations under common environmental conditions, often referred as 'common garden experiments' aim at minimizing non-genetic components of variation of the studied traits to reveal genetically based differences. Phenotypes can cover a variety of traits of different natures from morphometric measurements to quantification of gene expression. The "genetic architecture" of these traits can be obtained by QTL mapping.
- At the genome level, investigations are based on allele frequencies at given loci or – more directly – on DNA sequence data. In most cases, signature of selection on the genome provides indirect evidence, as alternative hypotheses cannot be totally ruled out. As a result, a question that often remains is to know if the observed differences between genotypes is really adaptive or results from other factors.

Linking phenotypes and genotypes at given loci is needed to provide direct evidence for response to local adaptation. However, cases for which direct links between observed variation for traits, DNA polymorphism and selective forces have been demonstrated remain rare. This is often due to the complex relationship linking DNA variation to the resulting phenotype as illustrated by (Dalziel *et al.*, 2009). Current progress in genomics of non-model organisms increases rapidly the number of well documented cases in marine species.

Common garden experiments: the phenotypic approach

Common garden experiments aim at disentangling environmental and genetic components of observed phenotypic differences. Under a "common garden", variations due to environmental factors are assumed to be minimized and the observed remaining variance is therefore presumed to be genetically based. The comparison of traits recorded on individuals originating from different locations or generations aims at identifying those putatively under differential selection. Such experiments are however strongly constrained by biological characteristics of the studied species and remain unfeasible for many marine species. In a first step, comparisons of specimens collected in natural populations can be performed, assuming that environmental differences encountered before the experiment will not significantly influence the recorded traits. In that case, a period of acclimatization to the common experimental condition is commonly used to reduce this bias. The efficiency of such acclimation period is however rarely assessed. Preferably, comparisons can be performed on progenies of individuals to be studied. By this way, common environment can be ensured but this approach implies that reproduction of the studied species is well mastered and that the development timing is rapid enough so that progenies reach the stage in which they will be phenotyped. This breeding step under controlled conditions minimizes influence from maternal or developmental effects originating from sampling the individuals directly in nature. Such a step may, however, strongly reduce the number of marine species where this approach can be applied. One additional difficulty related to this approach is that the tested progeny is representative of the studied population. This assumes that a large enough number of parental individuals are used with minimized variance of their reproductive success to avoid random drift.

Environmental conditions under which phenotypic characterization is undertaken can strongly influence the recorded traits. In case of significant genotype x environment interactions, different environmental conditions should be preferably tested. This will also allow estimating the genetic bases of plasticity of traits, which can be an important component of adaptiveness. Ideally, reciprocal transplant experiments will ideally reveal local adaptation. However, this is often most unpractical for marine species such as pelagic fish. The development and use of marine mesocosms for such studies remain challenging for most species and should be encouraged.

The development of transcriptomics studies, based on microarrays other high throughput approaches opened the possibility to score hundreds of traits as gene expression levels. In most cases, the genetic basis of these expression levels remains to be studied. In a first approach, this can be assessed by recording expression profiles of progenies resulting from crosses within and between individuals sampled in the studies populations. Intermediate levels of expression in "hybrid" progenies supporting additive variance genetic components. In a further step, heritability of expression level can be estimated in a similar way than morphometric traits.

Mapping adaptive traits within genomes: the QTL approach

Mapping of quantitative trait loci (QTLs) is commonly based on the analysis of experimentally controlled populations (e.g. F_2 progenies from a cross between inbred lines or more complex schemes involving on related individuals). Our working group reviewed QTL mapping in fisheries and aquaculture in 2008. When generation time is too long to allow such approaches, "whole-genome association studies" (WGAS) can be performed. These studies rely on linkage disequilibrium (LD) to detect an association between genotypes and phenotypes. The power and precision of these WGAS depend on the extent of LD in the studied population, which notably depends on its effective size (the smaller, the easier) and the number of loci scored. This is notably currently performed in cattle using SNP arrays (e.g. MacLeod *et al*., 2010). It such approaches, the use of phenotypic variation is a starting point and the statistical association of this variation with markers is the resulting goal. This approach therefore assumes that the adaptive traits have first been identified and measured.

Tracking the footprints of adaptation within genomes

The identification of variation at the DNA level of polymorphisms leading to presumed adaptive phenotypic variants has benefited in the recent years of the expansion of genomic technology. It should however be noted that, in several cases, early allozyme-based "classical" population genetics studied led to the identification of loci presumed to be under selection. For example, clinal variation of such markers along environmental clines can be indicative of local adaptation. They can however result from other evolutionary phenomena such as secondary contacts zones (e.g. in mussels: Boon *et al*., 2009).

Genome scans can be performed to identify loci or parts of the genome that appear under directional selection at the population level without phenotypic information. The detection of adaptive evolution at the molecular level essentially relies on indirect inferences. Direct inferences can of course be established if further information can be obtained regarding the functional role of those loci. "F_{st} ouliers" are defined as loci showing significant deviation from the other loci. Different methods and associated statistical tests have been proposed to identify outliers (Vitalis *et al.,* 2001; Foll and Gaggiotti, 2008).

It must be underlined here that a loci showing significantly higher (or lower) genetic differentiation and others is not necessarily under direct selection. They can be the result of associative effects, adaptive evolution leaving footprints on the pattern of neutral diversity by "hitchhiking". There are many similarities between the way demography and selection shapes genetic diversity. However selection only acts on the chromosomal neighbourhood of the site targeted while demography affects the whole genome. Population differentiation has an influence on hitchhiking: from "local effects" in the neighbourhood of favourable mutation to "global effects" (Bierne, 2010). As a result, scanning whole genomes (i.e. scoring large number of markers) is needed to discriminate between different causal factors of evolution.

Hierarchical testing is a way to increase confidence of candidate genes detected from genome scans. Starting out with a genome-wide distribution of genetic markers (preferably >100), one can perform genome scans to attain an initial set of candidates for selection (Figure 2.5.1). However, most outlier tests suffer from various levels of type I and II errors (Narum and Hess 2011). In order to further increase confidence in findings of natural selection at certain candidate markers, a range of "follow-up" approaches can be applied as far as data allows. First, if the underlying sequence of a

marker is known, annotation can be made to infer potential functions of gene regions underlying the genetic polymorphisms suggested outliers (Figure 2.5.1).

For populations genetically adapted to different environments (e.g. different temperature or salinity regimes) one would expect to find stronger correlations between important environmental drivers and the actual genes targeted by selection compared to neutral genes. An array of landscape genetics approaches allow to test for correlations between various environmental variables with each genetic marker independently (Joost *et al.* 2008; Coop *et al.* 2010). A pattern of stronger and more frequent landscape correlations for outlier markers than neutral markers will first of all suggest a potential evolutionary role of the particular variable, but also add confidence towards a true adaptive role for candidate markers showing such correlations (e.g. Narum *et al.* 2010). Increased support for a true adaptive role of candidate markers can be added if the study design allows for independent replication of tests (also referred to as parallelism in recent literature; Figure 2.5.1; Fraser *et al*. 2011).

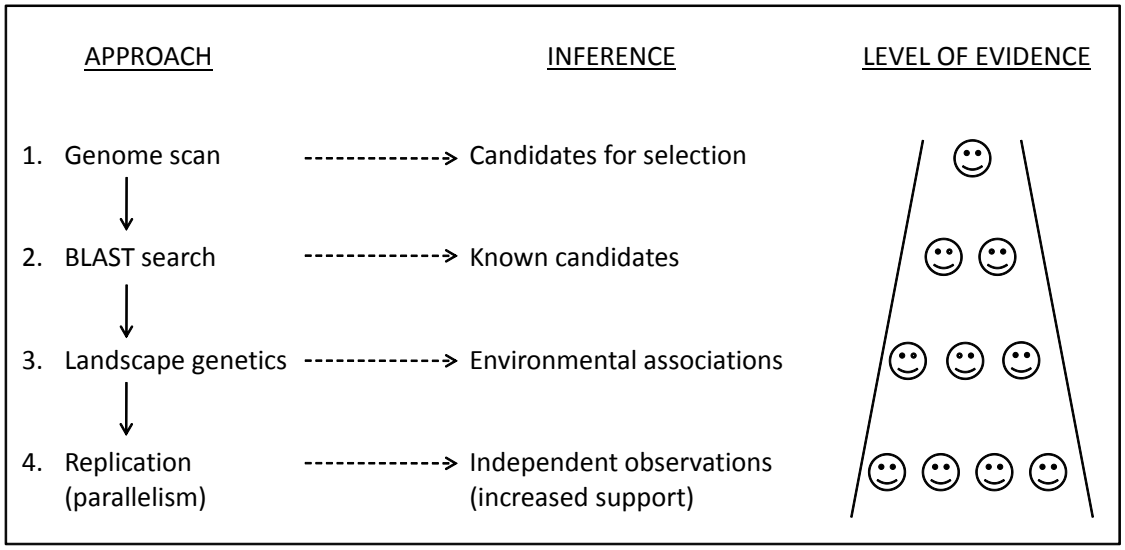

Figure 2.5.1. Conceptual diagram of a hierarchical approach for inferring in-direct evidence of selection at genetic markers (see text for more details).

Case studies and novel "model" species

Bradbury *et al*. (2010) followed the hierarchical approach outlined above for studying adaptations to temperature in Atlantic cod (*Gadus morhua*). They sampled cod populations along two independent temperature clines along either side of the Atlantic Ocean. Cod assemblages at either side of the Atlantic are expected to have followed independent evolutionary trajectories since they diverged between 100–150,000 years ago (Carr and Marshall 2008). However, the study by Bradbury *et al*. (2010) demonstrated a range of congruent outliers for divergent selection also showing strong correlations with temperature. BLAST annotations further suggested a range of different physiological processes to be associated with local temperature regimes. All together, this study found strong support for an adaptive role of the candidate genes underlying these congruent outliers. Currently, the study by Bradbury *et al*. (2010) demonstrates one of the most convincing findings of local adaption in a non-model marine fish following the indirect genotype based approach.

Understanding the functionality of evolution through genomic and transcriptomic analyses of commercial fish species is benefiting greatly from a few models. While reflecting scientific, historical (Wootton, 2009) and socio-economic determinants of choice, models have become accepted and have been shown to be very valuable. Current small fish models commonly used in ecology and evolution include the three-spined stickleback (*Gasterosteus aculeatus*), guppy (*Poecilia reticulata*) and mummichog (*Fundulus heteroclitus*). They are cited in the literature with about the same intensity, although reflect somewhat different scientific interests. For various reasons the small fish models of developmental developmental biology (the zebrafish *Danio rerio*) and medaka *Oryzias latipes*) have never attained a status of significance in eco-evolutionary research. Key traits for a model fish include generation time and lab footprint, experimental cost per animal, tolerance of broad environmental conditions, access to background biology (Bell and Foster, 1994), genomic tools (Oleksiak, 2010), the size of the research community, scientific literature (Östlund-Nilsson *et al*., 2007), tradition and experience.

Three-spined stickleback represents an outstanding model in eco-evolutionary research. It has contributed prominently to long standing questions such as the mechanisms of parallel evolution, sympatric speciation, directional selection, hybridization, the pace of evolution and eco-evolutionary dynamics. It is also a model in biomedical research for bone formation (Chan *et al*., 2010) and pigmentation (Miller *et al*., 2007). A nice case of the power of a small fish model is the pleiotropic effects of single gene changes (Pitx1- Peichel *et al*., 2001; Eda – Colosimo *et al*., 2005 and Kitlg – Miller *et al*., 2007). Small genomic changes may lead to large changes in phenotype, for example the presence of pectoral spines and lateral plates, and changes in pigmentation. Few cases have been documented in non-model species, such as the Pantophysin I gene in cod (Pogson and Mesa, 2004).

Whether selection acts on standing variation originate from a new mutation has been an issue for a long time (Schluter and Conte, 2009). The evolution of plateless stickelbacks in freshwater has its origin in the rare presence of an Eda allele in marine populations (Colosimo *et al*., 2005). Moreover, the process of divergence may act fast, as shown by the tolerance of cold. Fast evolution has also been proven in Atlantic silverside *Menidia menidia* (Conover and Munch, 2002) and guppies (van Wijk and Carvalho, pers. comm.), but remains largely to be documented in commercial fish (but see Jakobsdottir *et al*., 2011).

The maturation of technical developments in genomics and transcriptomics has led to considerable progress. In a short time genome scans, which look for signatures of selection in the genome, have expanded from not being possible to implement in a natural setting over a small set of markers (Makinen *et al*., 2008; Raeymaekers *et al*., 2009) to an almost full screening of the genome (Hohenlohe *et al*., 2010). The latter study found in a representation library of 100 different individuals signatures of genome-wide selection in freshwater and marine populations. Excellent knowledge of field gradients and experiments have facilitated the interpretation of the genomic data. At the moment the sequencing of 10 freshwater and 10 marine genomes collected worldwide is in progress by the Kingsley lab in California. This will lead to an even more detailed analysis of adaptation and selection, for example allowing understanding the functionality of selection.

While genome scans allow identifying gene regions and genes of interest, the functionality of these genes and gene regions remains largely unknown. Therefore research on a growing list of candidate genes has identified several interesting and

significant aspects (Colosimo *et al*., 2004; Miller *et al*., 2007; Wegner *et al* 2006). For example the regulation of the Pitx gene determines to a large extent the lateral plates of sticklebacks, of which the low plated morphotype has so remarkably colonized in parallel the rivers of the northern hemisphere. Body armour influences mobility and hence determines predation risk. Other strategies to identify candidate genes are transcriptomics, where the transcripts of the genes are studied for presence/absence, and more importantly up and down regulation (McCairns and Bernatchez, 2010). Increasingly a shift is occurring from single transcript screening (Peichel *et al*., 2001; McCairns *et al*. 2010), to full genome screening with micro-arrays (Leder *et al*., 2009) and currently also to whole genome transcriptome shotgun sequencing.

Understanding the full meaning of high throughput –omics approaches is backed by more traditional biology. Classical genetics including the heritability of traits (body armour - Mazzi *et al*., 2002; cold tolerance - Barrett *et al*., 2010, spine size - Barrett *et al*., 2008), and the mapping of traits (phenomics; Peichel *et al*., 2001, Shapiro *et al*., 2004). Behaviour (Pike *et al*., 2011) and comparative functional biology (Kitano *et al*., 2010) allow characterizing genomic changes, which often happen at the level of transcript regulation.

Marine fish and fisheries population genomics have largely developed in parallel with the findings in model species such as threespined stickleback (Nielsen *et al*., 2009 - review), although they have been constrained by good field data and experimental opportunities. A most striking finding is the molecular evidence for evolution induced through fishing. Although suspected for a long time (Rijnsdorp *et al*., 1996), confirmation is available from a remarkable allele shift in the pantophysin I gene of Icelandic cod populations over a period of only 55 years (Jakobsdottir *et al*., 2011).

With the arrival of affordable single-genome sequencing, the integration of information from genome, transcriptome, metabolome, physiology, life-history traits and ecology in field and experiment becomes increasingly feasible. Fish and fisheries biology has now more than ever before the means to understand the causes of evolution.

An elegant example of how insights in modes of selection and adaptive evolution can be obtained using genetic approaches is photic adaptation in the sand goby *Pomatoschistus minutus*. Polymorphisms were found for the rhodopsin gene RH1, initially selected as a candidate gene, which reflected water photic conditions rather than phylogeographic pattern. This suggests selection at the RH1 gene is involved in adaptation to light environments (Larmuseau *et al*., 2009). Additionally, synonymous and non-synonymous SNPs were compared between Baltic and North Sea regions. High levels of polymorphism were observed in the temporarily variable turbid conditions of the North Sea, whereas in the Baltic, where conditions are stable over time but photic conditions strongly differ between areas, signatures of stabilizing selection were observed. It is noteworthy that within one gene, synonymous and non-synonymous polymorphisms showed different modes of differentiation and this patterning could be used to infer both different modes of selection and demographic history (Larmuseau *et al*., 2010).

Recommendations

It is clear that monitoring of the genetic components of local adaptation in fisheries and aquaculture is required in view of changing selective pressures such as global change and fisheries induced evolution affecting productivity. Understanding of the dynamics of fitness, an important determinant of local adaptation in populations,

requires the integration of the various levels linking genotypic to phenotypic variation. Therefore we recommend:

1) Given the complexity of such undertaking, focusing on a few key aquatic species, providing well documented examples relevant to other species of interests for fisheries and aquaculture.

2) The incorporation of genome-wide genotyping as a tool in population studies.

3) Combining complementary approaches to minimize false positive markers and maximize the likelihood of identifying genes underlying adaptive processes in the wild.

4) The development of massive multi-trait phenotyping methods under natural and aquaculture conditions.

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