RESEARCH NOTE

Analysis of distribution of chromatin marks across "divergence islands" in three-spined stickleback (*Gasterosteus aculeatus*) [version 1; peer review: 2 approved with reservations]

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Abstract

The three-spined stickleback (*Gasterosteus aculeatus*) is a well-known model organism for studying adaptations to water salinity. In this work, we investigate the dynamics of an epigenetic landscape of water salinity adaptation using three chromatin marks: H3K27ac, H3K4me1 and H3K4me3. The choice of marks was determined by the fact that some adaptive genomic loci are situated in gene-free regions, suggesting their regulatory role as enhancers. Histone modifications seem to be a promising mechanism that could regulate such regions. Difference between histone modifications in sea and freshwater - both in genes and intergenic enhancers - may contribute to epigenetic plasticity of stickleback adaptation. As a result of this study, we found differential chromatin peaks in "divergence islands" at enhancer elements and promoters of genes, which are responsible for stress adaptation and homeostasis. However, a full genome study analysis is required to fully understand mechanism of adaptation to water salinity.

Keywords

divergence islands, chromatin marks, *Gasterosteus aculeatus*

Open Peer Review

Approval Status ? ?

	1	2
version 1		
20 Dec 2016	view	view
1. Alexey Ruzov , University of Nottingham, Nottingham, UK		
2. Ilkka Kronholm , University of Jyväskylä, Jyväskylä, Finland		

Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: The authors declare no conflict of interest.

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Introduction

The three-spined stickleback (*Gasterosteus aculeatus*) is a model organism that can be used to elucidate molecular mechanisms of adaptation to various salinities. In this work, we analyze epigenetic signatures that can be specific for various salinities. We study three chromatin marks H3K27ac, H3K4me1 and H3K4me3, which were chosen as these marks label active enhancers and promoters¹, in marine and fresh water sticklebacks in their natural habitats, as well as in foreign (change of salinity) environments. These marks were studied in 19 “divergence islands”², short genomic regions, which are highly diverged between marine and fresh water species. In the present study, 17 divergence islands overlapped in protein-coding genes, which are relevant to fresh water adaptation. In addition, we report the changes of histone modifications between marine and freshwater fish in promoters of protein-coding genes and enhancers.

Methods

Sample preparation and ChIP experiment

In this study, we used six marine and six fresh water sticklebacks, which were collected in august of 2015 from the White Sea (near the Pertsov White Sea Biological Station of Lomonosov Moscow State University, Murmanskaya Oblast, Russia) and Mashinnoe Lake (located near the village of Chkalvosky, Repuyblic of Karelia, Russia), respectively. The fish were placed for four days in various water tanks: half of the sticklebacks from each group was kept in the water with their natural salinity (FF and MM for fresh and marine water, respectively) and the other half was kept in a modified environment (i.e. three fresh water fish was placed in salty water (FM) and three marine fish were placed in fresh water (MF)). The live fish were transferred to the laboratory to Moscow.

For the chromatin immunoprecipitation (ChIP)-seq experiments, gills were collected from all 12 sticklebacks. Chromatin was prepared from gills, as described by Cell Signalling (<https://www.cellsignal.com/common/content/content.jsp?id=chip-agarose>) and ChIP was performed as described by Filion *et al.*³.

Bioinformatics analysis

Reads were aligned to gasAcu1 reference genome from the UCSC Genome Browser Gateway (<https://genome-euro.ucsc.edu/>) by Bowtie2 (<http://bowtie-bio.sourceforge.net/bowtie2/index.shtml>) with a “very-sensitive-local” parameter^{4,5}. For peaks calling we used MACS version 1.4.2⁶. For the intersection of peaks with divergence islands and genes we used bedtools version 2.26.0⁷.

Results

In our study, we determined between 7138 and 20828 histone modification peaks (Table 1). We selected histone modification peaks in “divergence islands” regions, which are highly divergent between marine and freshwater populations of sticklebacks². We observed that the majority (17 out of 19) of the islands showed the same chromatin marks (H3K4me1, H3K4me3 and H3K27ac) in fresh water species in their natural salinity (FF) and fresh water species placed into a marine environment (FM). The same was true for marine water species in their natural salinity (MM) and marine water species placed into a fresh water environment (MF). In addition, the majority of the islands (14 out of 19) showed the same histone modifications between the whole set of marine and fresh water species (MM+MF vs. FF+FM; Table 2).

Nevertheless, we found 3 out of 19 “divergence islands” demonstrating differential chromatin marks in cases of short-term adaptation to water salinity for fish placed into foreign environments. Interestingly, one “divergence island” gained H3K4me1 in the promoter of the RPTOR gene in FM. The other two islands gained H3K27ac and lost H3K4me1 in FM, suggesting their role as enhancers for genes outside the island. Also, two islands gained H3K4me3 and H3K4me1 at STC2 and PNPLA3 genes, respectively, in MF.

Finally, 5 out of 19 islands demonstrated differential histone modifications between fresh water species and marine species placed into fresh water (FF vs MF) (Table 3). In all these cases, MF gained a mark, which was absent in a FF. For example, H3K4me1 was gained in LRRC59 and BDH2 genes, which is involved in the

Table 1. Results of reads mapping and peaks calling. MM - marine species in natural environment; MF - marine species in fresh water; FF - fresh water species in natural environment; FM - fresh water species in marine water.

SamFile Name	All reads	Mapped reads	Ratio	Peaks
MMH3K27ac	16,319,162	13,242,763	81.14854795	11906
MMH3K4me1	15,805,735	12,244,401	77.46808991	13289
MMH3K4me3	7,354,166	6,872,442	93.44964473	12944
MFH3K27ac	16,917,583	9,700,338	57.33879361	13964
MFH3K4me1	12,773,849	10,303,455	80.66053544	20828
MFH3K4me3	14,099,289	7,836,369	55.57988775	18851
FFH3K27ac	14,567,425	11,367,244	78.0319377	9130
FFH3K4me1	12,243,305	10,737,653	87.70224216	8825
FFH3K4me3	15,176,697	9,476,326	62.43997623	14672
FMH3K27ac	14,850,800	11,887,597	80.04684596	12889
FMH3K4me1	13,213,512	10,426,786	78.91002786	7138
FMH3K4me3	14,512,630	11,658,225	80.33158015	15723

Table 2. Results of intersection of chromatin peaks with divergence islands and differential chromatin peaks analysis between MM and MF, and between FF and FM. "0" - no intersection; "1" - intersection. Regions with differential chromatin peaks are highlighted. MM - marine species in natural environment; MF - marine species in fresh water; FF - fresh water species in natural environment; FM - fresh water species in marine water. Differential peaks islands are highlighted in red.

Divergence islands coordinates			MM			MF			FF			FM		
Chromosome	Start	End	H3K27ac	H3K4me1	H3K4me3	H3K27ac	H3K4me1	H3K4me3	H3K27ac	H3K4me1	H3K4me3	H3K27ac	H3K4me1	H3K4me3
chrI	21,487,998	21,960,119	1	1	1	1	1	1	1	1	1	1	1	1
chrII	14,874,366	14,898,826	0	0	0	0	0	0	0	0	0	0	0	0
chrIV	12,803,780	12,881,296	1	1	1	1	1	1	1	1	1	1	1	1
chrIV	13,930,002	13,959,331	0	1	0	0	1	1	0	1	1	0	1	1
chrIV	19,811,922	19,914,666	1	1	1	1	1	1	1	1	1	1	1	1
chrIV	23,954,634	23,981,981	1	0	1	1	1	1	1	0	1	1	0	1
chrIV	26,016,955	26,166,536	1	1	1	1	1	1	1	1	1	1	1	1
chrV	2,482,209	2,501,295	1	1	1	1	1	1	1	0	1	1	0	1
chrVII	17,982,351	18,002,671	1	1	1	1	1	1	1	1	1	1	1	1
chrIX	8,521,935	8,537,559	1	1	1	1	1	1	1	1	1	1	0	1
chrIX	8,901,816	8,910,115	1	1	1	1	1	1	0	0	1	1	0	1
chrIX	9,208,158	9,227,809	0	0	0	0	0	0	0	0	0	0	0	0
chrIX	10334101	10353801	1	1	1	1	1	1	0	0	1	0	1	1
chrXI	5445757	5855124	1	1	1	1	1	1	1	1	1	1	1	1
chrXII	14338229	14358336	1	1	1	1	1	1	1	0	1	1	0	1
chrXII	16522028	16538810	0	0	0	0	0	0	0	0	0	0	0	0
chrXIX	2449903	2581858	1	1	1	1	1	1	1	1	1	1	1	1
chrXIX	14787904	14799088	0	0	0	0	0	0	0	0	0	0	0	0
chrXXI	5759879	7486635	1	1	1	1	1	1	1	1	1	1	1	1

Table 3 Results of intersection of chromatin peaks with divergence islands and differential chromatin peaks analysis between FM and MM, and between MF and FF. "0" - no intersection; "1" - intersection. Regions with differential chromatin peaks are highlighted. MM - marine species in natural environment; MF - marine species in fresh water; FF - fresh water species in natural environment; FM - fresh water species in marine water. Differential peaks islands are highlighted in red.

Divergence islands coordinates			FM			MM			MF			FF		
Chromosome	Start	End	H3K27ac	H3K4me1	H3K4me3	H3K27ac	H3K4me1	H3K4me3	H3K27ac	H3K4me1	H3K4me3	H3K27ac	H3K4me1	H3K4me3
chrI	21,487,998	21,960,119	1	1	1	1	1	1	1	1	1	1	1	1
chrII	14,874,366	14,898,826	0	0	0	0	0	0	0	0	0	0	0	0
chrIV	12,803,780	12,881,296	1	1	1	1	1	1	1	1	1	1	1	1
chrIV	13,930,002	13,959,331	0	1	1	0	1	0	0	1	1	0	1	1
chrIV	19,811,922	19,914,666	1	1	1	1	1	1	1	1	1	1	1	1
chrIV	23,954,634	23,981,981	1	0	1	1	0	1	1	1	1	1	0	1
chrIV	26,016,955	26,166,536	1	1	1	1	1	1	1	1	1	1	1	1
chrV	2,482,209	2,501,295	1	0	1	1	1	1	1	1	1	1	0	1
chrVII	17,982,351	18,002,671	1	1	1	1	1	1	1	1	1	1	1	1
chrIX	8,521,935	8,537,559	1	0	1	1	1	1	1	1	1	1	1	1
chrIX	8,901,816	8,910,115	1	0	1	1	1	1	1	1	1	0	0	1
chrIX	9,208,158	9,227,809	0	0	0	0	0	0	0	0	0	0	0	0
chrIX	10334101	10353801	0	1	1	1	1	1	1	1	1	0	0	1
chrXI	5445757	5855124	1	1	1	1	1	1	1	1	1	1	1	1
chrXII	14338229	14358336	1	0	1	1	1	1	1	1	1	1	0	1
chrXII	16522028	16538810	0	0	0	0	0	0	0	0	0	0	0	0
chrXIX	2449903	2581858	1	1	1	1	1	1	1	1	1	1	1	1
chrXIX	14787904	14799088	0	0	0	0	0	0	0	0	0	0	0	0
chrXXI	5759879	7486635	1	1	1	1	1	1	1	1	1	1	1	1

adaption to stress and homeostasis⁸, suggesting that these genes might be activated after the placement of marine fish into a fresh water environment. In addition, 6 islands out of 19 demonstrated differential histone modifications between marine and fresh water species placed in marine water, with both gains and losses of marks (Table 3). Among these, we found that H3K4me3 was gained at STC2 in FM* compared to MM*, H3K4me1 at LRRC59 and BDH2 in MM* compared with FM*, and H3K27ac at RPTR in MM* compared with FM*.

Conclusions

In this study, we analyzed the epigenetic profile of “divergence islands” with three chromatin marks, H3K4me1, H3K4me3 and H3K27ac. We report differential histone modifications that might be involved in the regulation of promoters and enhancers located in “divergent islands”, and therefore contribute to adaptation to water salinity. Furthermore, we found differential chromatin peaks at promoters of genes that are responsible for stress adaptation and homeostasis. The results of this study contributes to our understanding of molecular mechanisms of adaptation to water salinity. However, a full genome histone modification analysis is required in order to further understand these mechanisms of adaptation.

Data availability

Fastq files can be found in the SRA archive under accession number SRX2403902 (<https://www.ncbi.nlm.nih.gov/sra/?term=SRX2403902>).

Author contributions

SR designed the experiment. SZ and AM prepared ChIP-seq libraries. AS and EP carried out the research. AS wrote the manuscript.

Competing interests

The authors declare no conflict of interest.

Grant information

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Ilkka Kronholm

Centre of Excellence in Biological Interactions, Department of Biological and Environmental Sciences, University of Jyväskylä, Jyväskylä, Finland

The study by Sokolov et al. investigates changes in chromatin marks among saline and freshwater populations of sticklebacks. While I like the idea of the study and I do appreciate that this manuscript is intended as a short note, the current state of this manuscript is not suitable for indexing at the moment. The authors need to rewrite most of the manuscript as major revision is necessary. I've given some suggestions below.

Introduction

The introduction needs to be expanded so that the authors give some background on the system as well, there is plenty of previous research on the stickleback system that has been done and more of it needs to be cited here. Please also explain the ideas behind the divergence islands, give some information about these chromatin marks etc.

Methods

More information needs to be given about the chromatin immunoprecipitation experiments and the bioinformatic analysis. Currently the methods do not stand on their own. For example, at the moment the manuscript only states that MACS software was used for peak calling with no information how the method works or what parameters were used.

Results

I was wondering would it be helpful if the some of the results were shown as figures. Perhaps at least those peaks where differences were found.

Discussion

Currently the results are not really discussed at all. The authors should properly discuss their results. The authors found changes in certain genes, but the biological functions of those genes are barely mentioned or whether these are candidates for explaining adaptation to marine and freshwater environments.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 03 January 2017

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Alexey Ruzov

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In my opinion, the results on differential distribution of histone modifications in sea and freshwater three-spined sticklebacks may potentially be interesting. Despite this, I strongly believe that text of the manuscript is currently beyond the minimal standards required for paper indexing.

Specifically: the abstract does not describe main findings of the paper in sufficient detail; the introduction does not provide an adequate background information for the study but, instead, mainly repeats the abstract; any Discussion comparing the results with already available literature is missing; some crucial details of the methodologies used (e.g. which antibodies have been used for CHIP) are missing from Methods; some sentences in the text are difficult to understand.

I recommend the authors to rewrite the text of manuscript addressing these points.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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