Reviewer's Comments to Author

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Indication of family-specific DNA methylation patterns in developing oysters
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Reviewer 1 Reviewer's Comments to Author...

The role that epigenetic marking plays in the adaptation of marine invertebrates is certainly an interesting subject begging for mechanistic studies. The Roberts lab has taken a lead in this area, by producing the first evidence of DNA methylation for a marine invertebrate, the Pacific oyster.

This short MS extends earlier work by presenting original results of DNA methylation patterns for two maternal half-sib families. The inheritance and developmental patterns of DNA methylation are explored by sequencing of bisulfite-treated DNA from sperm and larvae at 3- and 5-days post-fertilization. Sequencing is done on six DNA libraries (2 families × 3 developmental stages), so the study lacks technical replication, which limits statistical inference. Using treated DNA from lamba phage, the efficiency of bisulfite conversion was determined to be 99.9%.

Although the number of sequenced cytosine bases is reported to be between 26 and 53 million across libraries, the MS reports very few numbers, five nodes in the Fig. 1 dendogram, 9 independent categories in Fig. 2, and 3 independent categories in Table 1. The proportion of differentially methylated loci between the two maternal half-sib families, 0.0046 (189/40,654), is of questionable significance, since it lacks replication and is not much higher than the estimate of bisulfite non-conversion. Even if this is accepted as a significant result, what does it mean and how might it be useful to other investigators?

It seems likely that these family differences simply reflect differences in the distribution of transposable elements between the two fathers, which is already known (Gaffney et al 2011 abstract J. Shellfish Res.; Zhang et al 2012 Nature). Thus, important questions about the functional roles of methylation remain unanswered, and the number of unmapped, differentially methylated loci between two fathers is provides no meaningful insight into population genetic causes or consequences.

The distributions, across four genomic features, of family- and developmental-specific differentially methylated loci are not significantly different (contingency chi-square = 4.93, 3 df, P=0.173), which seems to contradict conclusions in the last paragraph of the results section.

Preliminary remarks :
-Please help the reviewers ! by numbering lines of your text...
-"p3" means page 3.

Generals comments :

0-the topic is at the cutting edge of molecular biology and the application of such methods in oyster is exciting.

1-the experiments and analyses are of quality and are clearly described. I really appreciated the effort made by the authors to publish all the scripts on GitHub.
In addition, the discussion about the evolutionary role of methylation of transposable elements is well written (pros and cons arguments) with no over-interpretation.

2-the study describes tools/methodologies (e.g. GitHub) whose availability would be valuable for genetics and genomics investigators.

3-The results are original and adhere to most community standards for data availability and format;

Majors remarks/questions to authors :

p5, "Counts of oyster larvae were performed at 120 hpf to confirm normal development" => counting larvae is not enough to confirm normal development, biometric measures (such as length, width or area better). For example larvae at 120 hpf sampled on a 20µm screen are not fully comparable to larvae at 72 sampled on the same screen. You are not sampling the same part of larvae distribution. in other terms, at 72hpf you sampled between percentile 90 and 100 but at 120 hpf you sampled between percentile 20-100.

p6, "Only CpG loci covered by at least 3 sequenced reads were considered for further analysis." Do you assess the other value of coverage ? Does your results still hold with an higher coverage value ?

Do you remove PCR duplicates ? Or explained why it was not possible ?

p9, It is never mentioned how do you correct for multiple comparisons (Since the n of locii is 40,654...) when searching for DML using methylKit (bonferroni correction, Benjamini-Hochberg's method...) ?
In your Github (In [63] and In [69]), it seems that you filtered your loci on p-value, but this pvalue is not corrected for multiple hypothesis testing.

p10, In your Github (https://github.com/che625/olson-ms-nb/blob/master/BiGo_dev.ipynb, In [63]:) you never run whole analysis and quantify how many of the total variance is explained by family and development (Permanova) (for the interaction family*development you do not have enough df). Such analysis could strengthen your discussion.

p11, "Differentially methylated loci across families were distributed throughout the genome, though a higher proportion was found in transposable elements". This statement is inferred from the clustering procedures that use ward method on a Pearson correlation matrix. However, it is know that Ward’s method tend to find clusters with roughly the same number of observations in each cluster.
So how (un)balanced are each cluster in term of observation?
Or if linkage method gives similar results ?
Did you test with spearman correlation matrix which is more robust compare to Pearson distance ?