Distinguishing the putative U and V chromosomes of *Saccharina japonica* (Phaeophyceae) by cytogenetic mapping of sex-linked molecular markers

邮箱: m200100023@st.shou.edu.cn

Zhi Li[a], Yu Du[a] and Zhigang Zhou[b,*]

[a] Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources Conferred by Ministry of Education, Shanghai Ocean University, Shanghai 201306, China

[b] International Research Center for Marine Biosciences Conferred by Ministry of Science and Technology, Shanghai Ocean University, Shanghai 201306, China

Abstract

Saccharina japonica, an important economic species in brown algae, has a typical alternate life history of heteromorphic generations and UV sex determination system, but little is known about the sex chromosomes and sex determining regions (SDRs) in the *S. japonica*. In the present study, three female-linked (SJ-f_000170, MSj68-58-2 and FSMSJ-1294) sex-specific molecular markers, which were developed on the basis of the *Ectocarpus* sp. sex-determining regions. Discerning the putative U chromosome based upon the mono-color FISH profiles by these confirmed sex-linked markers. Bacterial artificial chromosome (BAC) clones were screened and sequenced from the constructed BAC libraries of *S. japonica* female gametophytes by female-linked markers. Combined with the result of different gene expression between male and female gametophytes of *S. japonica* using comparative transcriptome analysis, provide a preliminary identification of sex determining regions in *S. japonica* U chromosome。

Background

1. Some researches confirmed that an XY-like sex-determination system existed in *Saccharina japonica* (commonly referred to as kelp), and that the diploid sporophyte was XY type whereas the haploid female and male gametophytes were X- and Y-type, respectively.

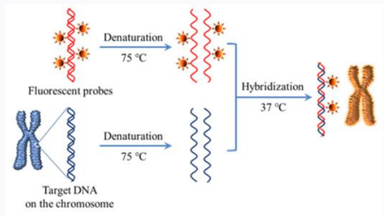
2. Such a contradictory view of whether there were sex chromosomes in *S. japonica* could not be resolved by cytogenetic evidence. The physical features, however, of the kelp chromosomes in number, size, and morphology as reviewed by Lewis (1996) limited further cytogenetic studies. Fortunately, this situation now has improved by applying the DNA-binding fluorochrome 4, 6-diamidino-2-phenylindole (DAPI) and the technique fluorescence in situ hybridization (FISH) to the observations on the kelp chromosomes (Liu et al. 2012).

3. By use of several strategies for identification of the sex-determining regions (SDRs) in *Ectocarpus siliculosus*, Ahmed et al. (2014) estimated that the male and female SDRs of *E. siliculosus* were 920 kbp and 929 kbp in size, respectively.

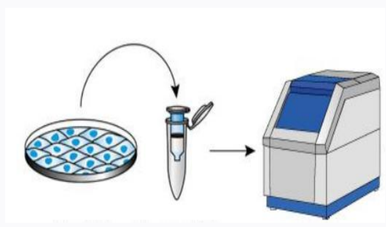
4. Lipinska et al. (2017) found that four and four genes were linked to *S. japonica* U and V chromosomes, respectively. Zhang et al. (2018) developed four new male gametophyte specific markers and one female gametophyte specific marker in *S. japonica* based on the SDR genes of *E. siliculosus*. As a result, these female-linked genes and molecular markers are expected to be co-located on the same U chromosome, whereas the male linked ones are to be on the same V chromosome of this kelp.

Materials and methods

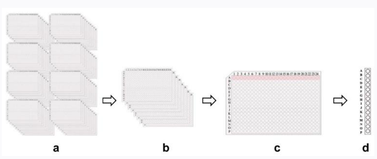
Culture male, female gametophyte and sporophyte of kelp



Use FISH technique to distinguish the putative U and V chromosomes of kelp

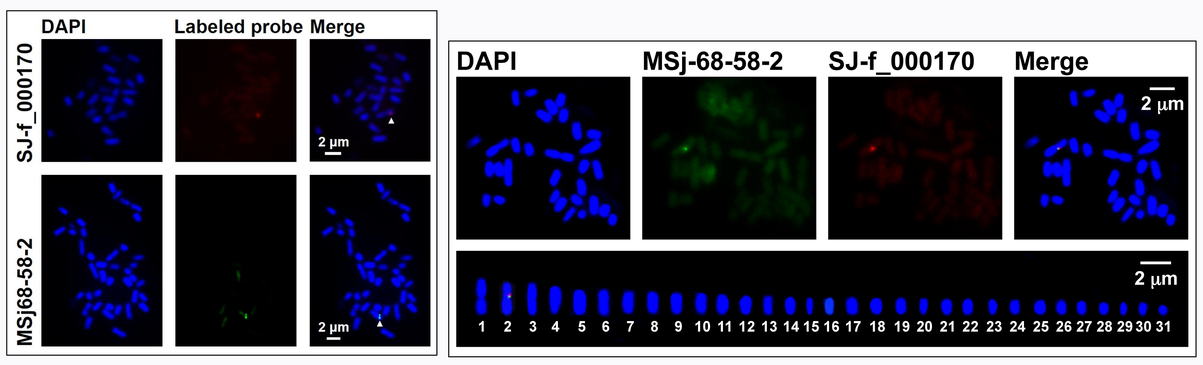


QRT-PCR for distinguishing the sex determining gene



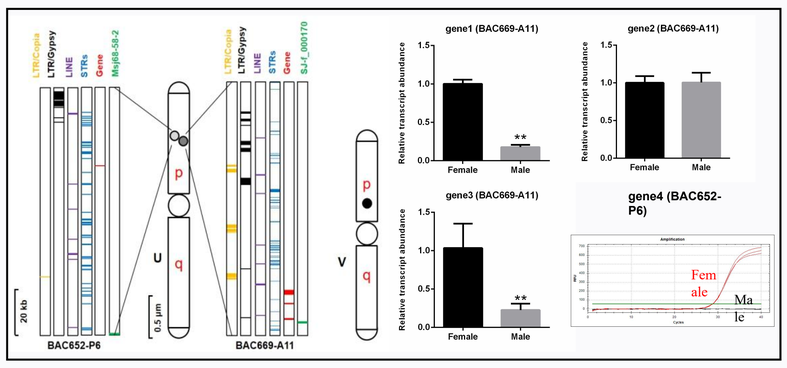
Use BAC library to establish the 3D screening pool and obtain the target BAC clones

Results and discussion



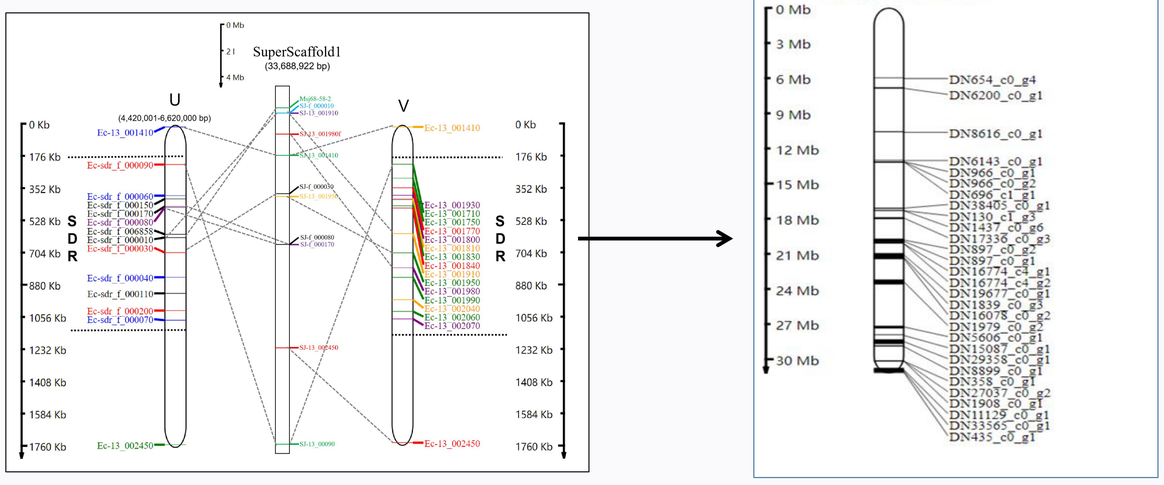
Monal- and Dual-color FISH mapping (upper panels) and idiogram (lower panel) of the sex-linked markers SJ-13_000170 (red) and MSj68-58-2 (green) on *Saccharina japonica* female gametophyte metaphase chromosomes counterstained with DAPI (blue)

- SJ-f_000170 and MSj68-58-2 was situated on Chromosome 2 of the kelp female gametophytes.
- These pieces of information demonstrated that the putative sex chromosome for the determination of female was not too conspicuous.



Schematic diagram of U and V chromosomes in *S. japonica* and expression comparison of three annotated genes between male and female gametophytes

- As a result of accumulation of more repeat sequences and poor gene density, the SDR of *S. japonica* U chromosome is expected to be expanded in size as compared to the U SDR of *Ectocarpus*.
- Gene 4 was referred to sex-specific genes. The other three genes, being transcribed differentially (for Genes 1 and 3) or similarly (for Gene 2) in abundance between the male and female gametophytes, were proposed to be gametologue pairs in *S. japonica* according to the termed definition by Coelho et al. (2018).



(a) Collinearity analysis between chromosome-level mapping of the *S. japonica* female gametophytes genome and high quality chromosome-level scaffolding of the *Ectocarpus* sp. genomes; (b) All sex-specific genes in the U sex chromosome (ie, supperscaffold1) of kelp which were verified by transcriptome.

- We emphasize that the Superscaffold1 in the middle of the figure is one pseudo-chromosome of the unpublished kelp genome. It can perfectly match the two BACs' sequences (both are greater than 99.8 % sequence coverage, no gaps).
- After comparison, we find that the physical distance between the two BACs is about 12 Mbp. In addition, one study (Lipinska et al. 2017) identified 59 V-linked scaffolds from the reference genome sequence (Ye et al. 2015) with cumulative size of 4.91 Mbp. Therefore, we firmly believe that the length of the SDR of the U chromosome of kelp must be more fold larger than that of *Ectocarpus* sp..

Conclusions

In a word, the present study offers novel insights into cytogenetic localization of sex-linked molecular markers, thus enabling us to distinguish the putative U sex chromosomes in *S. japonica*. In addition, the complete SDR sequence of the kelp U chromosome will be unveiled, once it is assembled by screening and sequencing of BAC clones. By that time, the true size and organization of the kelp female SDR will be brought to light.

上海海洋大学水产与生命学院