*Regular Article (Short Communication or Karyotype Report)*

Cytogenetic studies of sex chromosomes in *Ginkgo biloba* using fluorescent *in situ* hybridization

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Numbers of tables ( ), grayscale figures ( ), and color figures ( )

Short title: FISH analyses of sex chromosomes in *Ginkgo biloba*

**Summary** *Ginkgo biloba*, a gymnosperm, is a dioecious plant, but its sex chromosomes are morphologically difficult to distinguish. To cytogenetically clarify the sex chromosomes, fluorescent *in situ* hybridization (FISH) was performed using repetitive sequences. (Basically, Summary does not include details of the experimental conditions (reagent concentration, temperature, number of samples, number of experiments, *etc*.). (Times New Roman, 11 point)

**Keywords** Sex chromosome, Repetitive sequence, Fluorescent *in situ* hybridization, *Ginkgo biloba.*

*Ginkgo biloba* is known as a primitive gymnosperm, and the nut is used as food. When ginkgo is used as a roadside tree, it is necessary to plant male ginkgo trees to avoid the bad odor caused by falling fruits. Therefore, the sex determination of ginkgo is an important issue, and a number of DNA markers for sex determination have been identified so far. (Times New Roman, 11 point)

Materials and methods

*Plant materials (Italic)*

The experimental materials were three strains of *Ginkgo biloba.*

*Preparation of chromosome spreads*

The root tips were first treated with 0.3% 8-hydroxyquinoline at 20℃ for 3 h.

Results

It will be all right if you integrate the sections of Results and Discussion as “Results and discussion.” (Times New Roman, 11 point)

Discussion

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Supplementary information (If not, please omit this section)

Supplementary information including Table S1, Figs S1, S2, and Movie 1 is available online.

Author contributions

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If there are more than two authors, each author's contribution to the manuscript should be clearly stated.

Acknowledgments

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References

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Bennett, M. D. 1983. The spatial distribution of chromosomes. In: Brandham, P. E. and Bennett, M. D. (eds.). Kew Chromosome Conference II. Allen and Urwin Ltd., London. pp. 71-90.

Darlington, C. D. and Wylie, A. P. 1955. Chromosome Atlas of Flowering Plants. Allen and Unwin Ltd., London.

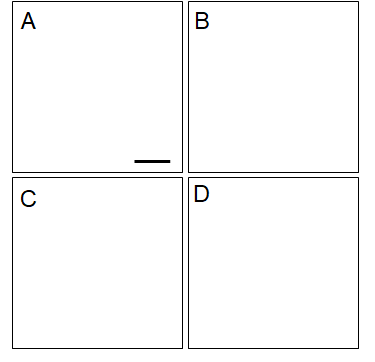
Harlen, K. M. and Churchman, L. S. 2017. The code and beyond: transcription regulation by the RNA polymerase II carboxy-terminal domain. Nat. Rev. Mol. Cell Biol. **18**: 263–273.

**Table 1. Variation of sex chromosomes in *Ginkgo biloba.***

Ecotype Sex chromosome length (μm) Spreads number

Tokyo 5.8±0.1a 34

Mean values with superscripted letters of each chromosome length are significantly different (*p* < 0.05) based on Tukey’s honestly significant difference comparisons. (Times New Roman, 10 point)



**Fig. 1.** **Fluorescent images of sex chromosomes in *Ginkgo biloba.*** (**A**) Metaphase spreads of mitotic chromosomes. Scale bar = 2 μm. (Times New Roman, 10 point)

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