Jpn. J. Phycol. (Sôrui) 42: 401–406, December 10, 1994

Mapping of centromere distances of light green and light red genes in *Porphyra yezoensis* (Rhodophyta, Bangiales)

Jin Hamada*, Jong-Ahm Shin** and Akio Miura**

*Department of Community Medicine, Faculty of Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama, 930-01 Japan

**Department of Bioscience and Biotechnology, Faculty of Engineering, Aomori University, 2-3-1 Kohbata, Aomori, 030 Japan

Hamada, J., Shin, J.-A. and Miura, A. 1994. Mapping of centromere distances of light green and light red genes in *Porphyra yezoensis* (Rhodophyta, Bangiales). Jpn. J. Phycol. **42**: 401–406.

Centromere distances (CDs) of thallus-colour genes, light green (*lg*) and light red (*lr*) types in *Porphyra yezoensis* were calculated by the data of Ohme *et al.* (1986) and Ohme and Miura (1988) according to the formula of Whitehouse (1949) and Perkins (1949). The CDs were 21.6 and 14.1 centimorgan for *lg* and *lr*, respectively. The present study discussed the CDs calculated from monohybrid or dihybrid crosses and different viabilities of chimeric thallus for several colour mutant genes.

Key Index Words: Bangiales—centromere distance—genetics—mapping—Porphyra yezoensis *Rhodophyta—tetrad analysis*

Mapping of CD in ordered tetrad was established in Ascomycete *Neurospora crassa* (Beadle 1945). In *Porphyra yezoensis*, Ohme *et al.* (1986) and Ohme and Miura (1988) showed that the four-cell germlings from conchospores were equivalent to the ordered tetrads. The latter revealed that the genes for green type thallus colour (abbreviation: phenotype is G, gene symbol is *g*) and red colour type (R, *r*) were located in the different arms of the same chromosome, i.e., linkage group I. The map distances of *g* and *r* were demonstrated to be 15.9 (15.8 in Ohme and Miura 1988) and 17.9 centimorgan apart from the centromere, respectively (Fig. 1). Ohme and Miura (1988) showed that the other colour mutant genes, light green type (LG, *lg*) and light red type (LR, *lr*), belonged to two different linkage groups. The chromosomes which carry *lg* and *lr* were coined as linkage groups II and III, respectively (Fig. 1). Ohme and Miura (1988), however, did not show the data of the crosses between W × LG and W × LR, nor CDs of *lg* and *lr*.

In the present study, we could calculate the CDs of *lg* and *lr* from their data. We applied a formula which deals with a relation between the proportion of tetratype tetrads and that of the second division segregations (SDS) in dihybrid crosses. However, precise mapping of the genes in *P. yezoensis* is difficult, because there may be different viabilities and development among several colour mutants in a chimeric thallus, and the resultant trouble in the detection of SDS from first division segregation (FDS) in some cases. In spite of these difficulties at the present time, we hope the study of *P. yezoensis* become more popular since this alga is important for Japanese people traditionally.

Materials and Methods

Genetical data of colour mutant genes in *Porphyra yezoensis* Ueda were cited from Miura (1985), Ohme *et al.* (1986), Ohme and Miura (1988), and Niwa *et al.* (1993).

CDs of colour mutant genes in *P. yezoensis* were calculated according to the formula of Whitehouse (1949) and Perkins (1949): In case of dihybrid, when two allelomorphic genes, *A/a* and *B/b*, are located in different chromosomes each other, and *Ab* and *aB*, for instance, are crossed as parents, parental

Table 1. Proportion of parental ditype (PD), non-parental ditype (NPD) and tetratype (TT) tetrads in the F_1 progenies of the cross between two allelomorphic genes, *A/a* and *B/b*, which are located in different chromosomes each other.

Type of cross-over*	PD	NPD	TT	Proportion**
No cross-over	1/2	1/2	0	$(1-x)\quad(1-y)$
Cross-over in 1 locus	0	0	1	$x(1-y)+y(1-x)$
Cross-over in 2 loci	1/4	1/4	1/2	xy

* Cross-over between a gene(s) and the centrometer(s).
** x and y are the proportion of SDS at the *A* and *B* loci, respectively.

ditype (PD), non-parental ditype (NPD) and tetratype (TT) tetrads are produced in the F_1 progenies. As shown in Table 1, if x and y were postulated to be the proportion of SDS at the *A* and *B* loci, respectively, the proportion of TT, p, in the F_1 progenies is as follows (Whitehouse 1949, Perkins 1949):

$$p=x(1-y)+y(1-x)+xy/2$$
$$=x+y-3xy/2, \cdots\cdots \text{Form. 1}$$

Results

If it was assumed that the proportion of tetratype tetrads for the cross between G and LR is **q**, and that of SDS for G and LR are **g** and **lr**, respectively, Form. 1 is replaced by the equation as follows:

$$q=g+lr-3g\cdot lr/2 \cdots\cdots \text{Eq. 1}$$

The left side is able to be calculated from the cross, G × LR (Table 2), as below:

$$q=346/(210+187+346)=0.466$$

When the value of 0.318 (Table 3) is applied to **g**, Eq. 1 becomes as follows:

$$0.466=0.318+lr-3\cdot 0.318\cdot lr/2$$
$$lr=0.282$$

Therefore, CD of *lr* is:

$$0.282/2\times 100=14.1 \text{ (centimorgan)}$$

Then, concerning the cross between LG and R in Table 2, if we assume that the proportion of the tetratype tetrads as **s**, and that of SDS for R and LG as **r** and **lg**, respectively, Form. 1 is replaced by the equation as follows:

$$s=r+lg-3r\cdot lg/2 \cdots\cdots \text{Eq. 2}$$

If the values in Table 2 were applied to Eq. 2,

$$\text{left side}=1386/(557+539+1386)=0.558.$$

When **r** is replaced by 0.359 (Table 3), Eq. 2 becomes as follows:

$$0.558=0.359+lg-3\cdot 0.359\cdot lg/2$$
$$lg=0.432$$

Therefore, CD of *lg* becomes as below:

$$0.432/2\times 100=21.6 \text{ (centimorgan)}$$

Next, we could go over these calculations by applying the data of LR × LG (Table 2) to the following equation as below:

$$t=lr+lg-3lr\cdot lg/2 \cdots\cdots \text{Eq. 3}$$

where **t** is the proportion of tetratype tetrads in the cross, LR × LG.

Table 2. Frequencies of parental ditype (PD), non-parental ditype (NPD) and tetratype (TT) tetrads, and the ratio of PD to NPD in the F_1 thalli of the crosses among light red (LR), light green (LG), red (R) and green (G) (Ohme and Miura 1988).

Crosses	PD	NPD	TT	PD/NPD
G × LR	210	187	346	1.12
LG × R	557	539	1386	1.03
LR × LG	153	170	401	0.90
Eptd LR × LG*	169.778	169.778	384.444	1.00

* Expected values from the cross, LR × LG. See the text.

Table 3. Types of coloured thalli and their frequencies in the F_1 progenies of the crosses, green type (G) × wild type (W), and wild type (W) × red type (R) (Ohme et al. 1986).

G (♀) × W (♂)			W (♀) × R (♂)		
Colour types		(TOS)*	Colour types		(TOS)*
Single colour			Single colour		
W	186		W	26	
G	114		R	24	
Chimera		2884 (FDS)	Chimera		1069 (FDS)
W+G	2584		W+R	1019	
W+G+W	691		W+R+W	280	
G+W+G	637	1344** (SDS)	R+W+R	316	598 (SDS)
W+G+W+G	16		W+R+W+R	2	
Proportion of SDS		0.318			0.359
CD (centimorgan)		15.9**			17.9

* Types of segregations: first (FDS) or second (SDS) division segregation.
** In Ohme and Miura (1988), CD of *g* was calculated as 15.8, because the number of SDS was totalled as 1334.

left side
$=401/(153+170+401)=0.554$

right side$=0.282$
$+0.432-3\cdot0.282\cdot0.432/2=0.531$

Thus.

left side≒right side.

Here, x^2 was calcuated to confirm if the left side value fitted to the right. The expected values were put on the basis that t be 0.531, and the ratio of PD/NPD be 1.0.

$$x^2=[(153-169.778)^2/169.778]+[(170-169.778)^2/169.778]+[(401-384.444)^2/384.444]=2.371^{N.S.}\ (P>0.3)$$

Therefore, CDs of 14.1 and 21.6 for *lr* and *lg*, respectively, were consistent with each other. The linkage map of the four colour mutants of *P. yezoensis* is shown in Fig. 1.

Discussion

In *Porphyra yezoensis*, meiosis starts when a conchospore germinates and is completed at the four-cell conchospore germling stage (Ohme *et al.* 1986, Ohme and Miura 1988). The tetrads after the meiosis arranged linearly in the haploid leafy thallus, and CDs were determined by the tetrad analysis (Ohme and Miura 1988, Niwa *et al.* 1993).

In the present study, CDs of two thallus-colour genes, *lg* and *lr*, were calculated according to the formula of Whitehouse (1949) and Perkins (1949). There was no contradiction among these values, statistically. Therefore, CDs of *lg* and *lr* thus obtained were estimated to be reliable.

On the contrary, Miura (1985) reported the results of reciprocal crosses between the wild type and LG (Table 4). If the chimeric thalli of W+LG+W, LG+W+LG, and W+LG+W+LG were supposed to be SDS, the CD of *lg* is calculated to be 16.0 or 12.3 by the crosses of W (♀) × LG (♂) or LG (♀) × W (♀), respectively. These values differed statistically from the one obtained in the present study (21.6 centimorgan; $x^2=48.209^{**}$ and 275.899^{**} for W (♀) × LG (♂) and LG (♀) × W (♂), respectively). The discrepancy means that three of the other colour type thalli (W, LG, and W+ LG) in Table 4 should have SDS which could not be detected under the conditions they observed.

A similar phenomenon was observed in the reciprocal crosses between the wild type and LR (Table 5, Miura 1985). If the chimeric thalli of W+LR+W, LR+W+LR, and W+LR+W+LR were supposed to be SDS, CD of LR (*lr*) is calculated as 12.5 and 12.0

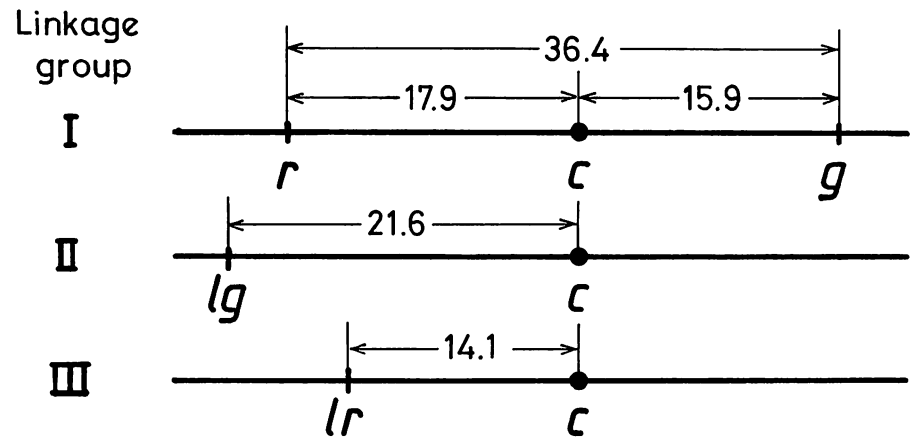

Fig. 1. Linkage map of colour mutant genes in *Porphyra yezoensis* Ueda. The units are in centimorgan. *r*: red type, *g*: green type, *lg*: light green type, *lr*: light red type, and *c*: centromere.

centimorgan from the crosses of W (♀)×LR (♂) and LR (♀)×W (♂), respectively. However the difference between these values and that obtained in the present study (14.1 centimorgan) was small, the x^2 values were significant (3.979* and 12.869** for W (♀)×LR (♂) and LR (♀)×W (♂), respectively). The value obtained from the dihybrid was also bigger than that obtained from the monohybrid. The other three colour type thalli (W, LR, and W+LR) may have undetectable thalli which have carried out SDS.

The discrepancy between the data from the monohybrid and those from dihybrid may be originated from unequal growth of four tetrad cells. To overcome this problem, dihybrid, trihybrid, or multihybrid crosses as well as the monohybrid crosses are desirable. Although the discrimination of the colour order between from the top and from the base of a thallus have not been recorded except in Miura and Ohme-Takagi (1994), it is necessary for the genetical and developmental studies of *P. yezoensis*. According to their data, it is presumable that there were some differences in viability among various colours of tetrads. If the order of from the top or from the base were distinguished, the development of thalli, vigour of each colour among the tetrads and

Table 4. Types of coloured thalli and their frequencies in the F_1 progenies of the reciprocal crosses between the wild type (W) and light green type (LG) (Miura 1985).

Colour types	Number of F_1 thalli			
	W (♀)×LG (♂)		LG (♀)×W (♂)	
	Emnt*	Eptd**	Emnt*	Eptd**
Single-coloured thallus				
W	64		133	
LG	46		84	
Chimeric thallus		529.944 (FDS)***		1100.784 (FDS)***
W+LG	525		1246	
W+LG+W	146		234	
LG+W+LG	136	403.056 (SDS)***	241	837.216 (SDS)***
W+LG+W+LG	16		0	

* Experimental data.
** Expected values supposing that CD of *lg* as 21.6.
*** FDS and SDS represent first and second division segregation, respectively.

Table 5. Types of coloured thalli and their frequencies in the F_1 progenies of the reciprocal crosses between the wild type (W) and light red type (LR) (Miura 1985).

Colour types	Number of F_1 thalli					
	W (♀) × LR (♂)			LR (♀) × W (♂)		
	Emnt*	Eptd**		Emnt*	Eptd**	
Single-coloured thallus						
W	77			129		
LR	45			24		
Chimeric thallus		598.094 (FDS)***			1061.922 (FDS)***	
W + LR	502			971		
W + LR + W	107			212		
LR + W + LR	80	234.906 (SDS)***		139	417.078 (SDS)***	
W + LR + W + LR	22			4		

* Experimental data.
** Expected values supposing that centromere distance of *lr* as 14.1.
*** For FDS and SDS, see the legend to Table 4.

genetics of the thalli will be disclosed more precisely.

Recently, Niwa *et al.* (1993) studied the thallus-colour of violet (V) and demonstrated that the violet gene (*v*) was located in a different chromosome from the linkage group I. They located *v* in 7.78 or 9.84 centimorgan apart from the centromere (Table 6). Their genetical results, however, have some contradictions. For instance, the difference in recombination frequencies obtained from the reciprocal crosses was not small (Table 6) and in the cross of V (♀) × G (♂), the ratio of PD to NPD is greatly apart from 1 ($x^2=9.511$*, Table 7). As the proportion of tetratype tetrad in the cross of G (♀) × V (♂) be 0.434 (Table 7) and that of the SDS of *g* be 0.318 (Table 3), **v**, the proportion of the SDS of the gene, *v*, becomes as follows:

$$0.434=0.318+\mathbf{v}-3\cdot0.318\cdot\mathbf{v}/2$$
$$\mathbf{v}=0.243$$

Therefore, CD of gene *v* becomes as below:

$$0.243/2\times100=12.2 \text{ (centimorgan)}.$$

Here, the value obtained from the dihybrid (12.2 centimorgan) was also bigger than those obtained from the monohybrid. The difference might be due to the higher detectable level in dihybrid than in monohybrid.

As to the detectability of thalli which carried out SDS from those of FDS, there may be differences among the colour mutants. The differences might be due to the different viability level among them. For instance, red colour type which produces nealy the same number of W and R in the cross W (♀) × R (♂) (Table 3), may have higher viability than green colour type which produced less number of G than W in the cross, G (♀) × W (♂) (Table 3).

As *P. yezoensis* has only three haploid chromosome (Yabu and Tokida 1963, Migita 1967, Yabu 1969, Kito 1978, Ohme and Miura 1988, Tseng and Sun 1989), it is relatively easy to map any genes to each chromosome and to have a bird's eye view about the gene configuration. Mapping of colour mutant genes is interesting and important not only from genetical view point, but also from physiological one. The genetical studies of other colour mutant genes are now in progress in our laboratory.

Table 6. Frequencies of first division segregation (FDS), second division segregation (SDS) and centromere distance (CD) of the gene, *v*, from the reciprocal crosses between the violet type (V) and the wild type (W) (Niwa *et al.* 1993).

Cross (♀ × ♂)	FDS	SDS	CD
W × V	982	181	7.78
V × W	873	214	9.84

Table 7. Frequencies of parental ditype (PD), non-parental ditype (NPD) and tetratype (TT) tetrads, ratio of TT to the total tetrads and the ratio of PD to NPD in the F_1 thalli of the reciprocal crosses between violet type (V) and green type (G) thallus colour (Niwa *et al.* 1993).

Cross (♀ × ♂)	PD	NPD	TT	TT/total	PD/NPD
V × G	342	266	549	0.475	1.29
G × V	296	287	447*	0.434	1.03

* The number was corrected from original number, 477.

Acknowledgements

The authors thank Dr. Kyoko Hayashi of Faculty of Medicine, Toyama Medical and Pharmaceutical University and Dr. Masaru Ohme-Takagi of National Institute of Bioscience and Human Technology, Tsukuba, for their critical reading of the manuscript.

References

Beadle, G. W. 1945. Genetics and metabolism in *Neurospora*. Physiol. Revs. **25:** 643–663.

Kito, K. 1978. Cytological studies on genus *Porphyra*. Bull. Tohoku Reg. Fish. Lab. **39:** 29–84. with Pl. I-XLIV. (in Japanese with English Abstract).

Migita, S. 1967. Cytological studies on *Porphyra yezoensis* Ueda. Bull. Fac. Fish. Nagasaki Univ. **24:** 55–64.

Mirura, A. 1985. Genetic analysis of the variant color types of light red, light green and light yellow phenotypes of *Porphyra yezoensis* (Rhodophyta, Bangiaceae). pp. 271–284. *In* Hara, H. (ed) Origin and Evolution of Diversity in Plants and Plant Communities. Academia Scientific Book. Tokyo.

Miura, A., Ohme-Takagi, M. 1994. Mendelian inheritance of pigmentation mutant types in *Porphyra yezoensis* (Bangiaceae, Rhodophyta). Jpn. J. Phycol. **42:** 83–101. (in Japanese with Engblish abstract).

Niwa, K., Miura, A., Shin, J.-A. and Aruga, Y. 1993. Characterization and genetic analysis of the violet type pigmentation mutant of *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta). Korean J. Phycol. **8:** 217–230.

Ohme, M., Kunifuji, Y. and Miura, A. 1986. Cross experiments of the color mutants in *Porphyra yezoensis* Ueda. Jpn. J. Phycol. **34:** 101–106.

Ohme, M. and Miura, A. 1988. Tetrad analysis in conchospore germlings of *Porphyra yezoensis* (Rhodophyta, Bangiales). Plant Science **57:** 135–140.

Perkins, D. D. 1949. Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:** 607–626.

Tseng, C. K. and Sun, A. 1989. Studies on the alternation of the nuclear phases and chromosome numbers in the life history of some species of *Porphyra* from China. Bot. Mar. **32:** 1–8.

Whitehouse, H. L. K. 1949. Multiple-allelomorph heterothallism in the fungi. New Phytol. **48:** 212–244.

Yabu, H. 1969. Observation on chromosomes in some species of *Porphyra*. Bull Fac. Fish. Hokkaido Univ. **19:** 239–243.

Yabu, H. and Tokida, J. 1963. Mitosis in *Porphyra*. Bull. Fac. Fish. Hokkaido Univ. **14:** 131–136 with pl. I–VI.

濱田　仁*・申　宗岩**・三浦昭雄**：紅藻スサビノリ (*Porphyra yezoensis*) の 2 個の色素変異遺伝子，明緑色型 (light green type gene) と明赤色型 (light red type gene) の動原体距離

スサビノリ (*Porphyra yezoensis*) の色素変異体の明緑色型 (light green type) と明赤色型 (light red type) 遺伝子の動原体からの距離が，各々21.6と14.1センチモルガンであることを，Ohme *et al.* (1986) と Ohme and Miura (1988) の資料に基づき，Whitehouse (1949) と Perkins (1949) の方程式を用いて明らかにした。また，スサビノリの減数分裂後，四分子が増殖して葉状体を形成する際には，色素変異体の色の違いにより増殖力に差があることが，色の違う葉状体の交配によって出来たキメラ状葉状体の種類とその出現頻度から推定された。従って，スサビノリで遺伝子地図を作成する際には，単性雑種よりも両性雑種か三性雑種を用いる方が望ましいと考えられる。
(*930-01 富山市杉谷2630　富山医科薬科大学医学部保健医学教室，**030 青森市幸畑2-3-1　青森大学工学部生物工学科)

(*Received August 11, 1994. Accepted October 10, 1994*)