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Cross experiments of the color mutants in *Porphyra yezoensis* UEDA*

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Cross experiments have been achieved among the wild type, the red type mutant and the green type mutant in *Porphyra yezoensis*. Both the mutants were recessive to the wild type. The heterozygous conchocelis between the red type and the green type mutants was the wild type, indicating that the mutants complemented each other, and the yellow phenotype newly appeared as a result of recombination between the loci of the red and the green type mutants. Most of the F_1 thalli (92.9-99.5%) of the heterozygous conchocelis were sectorially variegated chimeral thalli composed of various combinations of color sectors which arose from meiotid segregation. Conchospores are assumed to be released during meiotic prophase to segregate haploid phenotype during their germination, and this leads to the formation of variegated chimeral thalli in *P. yezoensis*.

Key Index Words: *Chimeral thallus*; *color mutant*; *cross experiment*; Porphyra yezoensis.

In recent years red type mutant thalli or sectorially variegated chimeral thalli have been found in cultivated populations and laboratory cultures of *Porphyra yezoensis* UEDA (MIURA 1984). KOBARA *et al.* (1976) established the green type strain and ARUGA and MIURA (1984) characterized the red type and the green type strains of *P. yezoensis*. KIKUCHI *et al.* (1979) reported chemical nature of phycobilins of the color mutants of *P. yezoensis* from our laboratory. ARUGA and MIURA (1984) have made clear their characteristics by comparing *in vivo* absorption spectra. Comparative studies on the growth and photosynthesis of the mutants of *P. yezoensis* have been achieved (KATO and ARUGA 1984). As to other species of seaweeds, VAN DER MEER and his coworkers reported the genetic studies on the pigmentation mutants of *Gracilaria* (VAN DER MEER 1977, 1978, 1979a, b, 1980, VAN DER MEER and BIRD 1977, VAN DER MEER and TODD 1977) and a study of the life history of *Palmaria palmata* with a pigmentation mutant (VAN DER MEER and TODD 1980). However, there has been no genetical approach to *P. yezoensis*. Since the mutant strains are useful markers for genetic studies on *P. yezoensis*, crosses were performed for an initial study in this species. We report here the results of the crosses among the wild type and the mutants.

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Materials and Methods

The wild type strain (W, strain number U-51) was isolated from a cultivated population of *Porphyra yezoensis* at Ushigome,

Kisarazu City, Chiba Prefecture, in 1974 and the red type strain (R, F-6) was established from a red type mutant thallus isolated from a cultivated population of *P. yezoensis* at Shitazu, Futtsu City, Chiba Prefecture, in 1974. The green type strain (G, C-0 giant) was established by KOBARA *et al.* (1976). Laboratory cultures of thalli and conchocelis were carried out as described by KATO and ARUGA (1984). Crosses were performed by coculturing the marginal pieces of different phenotype thalli. When carposporangia were formed on the thallus piece, each piece was separated and cultured until carpospores were released. Carpospores collected from the crossed thallus piece were cultured in a petri dish and were separated one by one when they grew into a conchocelis colony 1 mm in diameter. Color types of the progeny thalli produced from the crossed conchocelis were determined with thalli 0.5–1.5 mm in length.

Results and Discussion

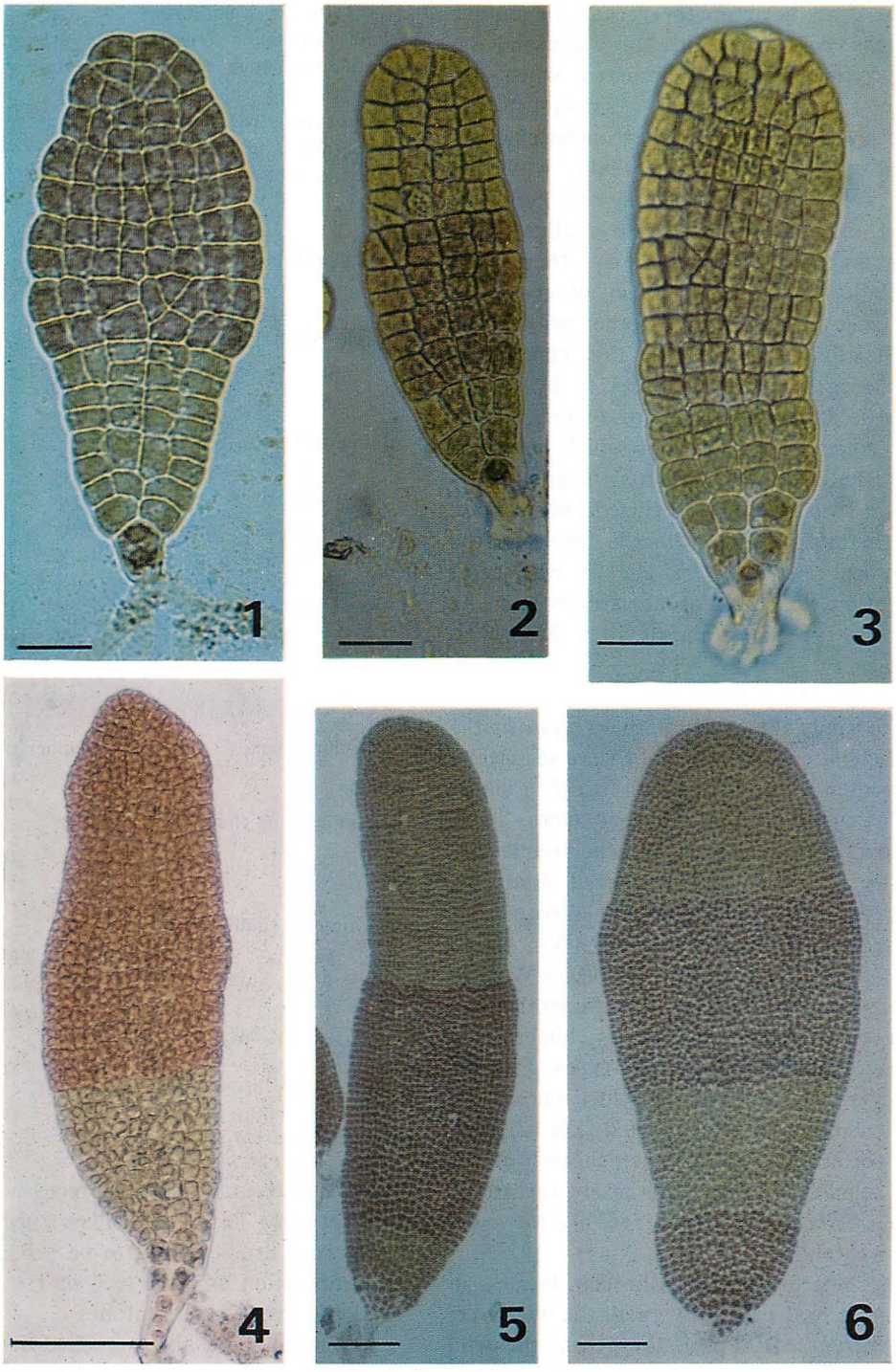
Crosses of the wild with the red and the green types

To characterize the mode of transmission of the mutants, the red type and the green type mutants were crossed with the wild type. In the cross experiments, we regarded the thallus from which carpospores were taken as female parent. Table 1 shows the results of these crosses. In the cross between the red type mutant and the wild type, F_1 conchocelis were of all the wild type when the wild type was female. Some conchocelis produced only the wild type thalli, while others produced both the wild and the red types in F_1 thallus phase. In the reciprocal cross, when the red type mutant was female, both the red and the wild type conchocelis occurred. The red type conchocelis produced only the red type thalli, while the wild one segregated the wild and the red types in F_1 thallus phase.

Table 1. Results of the reciprocal crosses of the wild with the red and the green type mutants of *Porphyra yezoensis*. W, wild type; R, red type; and G, green type. (* Female parent shown first.)

Cross*	F_1 conchocelis	F_1 thalli	Putative cross combination
W × R	W	W	W × W (self.)
	W	W, R (chimeral thalli)	W × R (cross.)
R × W	R	R	R × R (self.)
	W	W, R (chimeral thalli)	R × W (cross.)
G × W	G	G	G × G (self.)
	W	W, G (chimeral thalli)	G × W (cross.)

Figs. 1-6. Various types of sectorially variegated chimeral thalli which arose from heterozygous conchocelis in *Porphyra yezoensis*. Scale bar 30 μm in Figs. 1-3; 0.1 mm in Figs. 4-6. Fig. 1. A two-sectorial chimeral thallus composed of the green and the wild type sectors arisen from the heterozygote of the wild and the green types. Fig. 2. A three-sectorial chimeral thallus composed of the green and the wild type sectors arisen from the heterozygote of the wild and the green types. The green type sector is repeated. Fig. 3. A three-sectorial chimeral thallus composed of the yellow, the red and the green type sectors from the apex to the base of the thallus arisen from the heterozygote of the red and the green types. Fig. 4. A two-sectorial chimeral thallus composed of the red and the



green type sectors arisen from the heterozygote of the red and the green types. Fig. 5. A three-sectorial chimeral thallus composed of the green and the red type sectors arisen from the heterozygote of the red and the green types. The green type sector is repeated. Fig. 6. A four-sectorial chimeral thallus composed of the yellow, the wild, the green and the red type sectors from the apex to the base of the thallus arisen from the heterozygote of the red and the green types.

In the cross between the green type mutant and the wild type, both the green and the wild type conchocelis occurred when the green type was female. Similar to the prior cross, the green type conchocelis produced only the green type thalli, while the wild type conchocelis produced both the green and the wild types in F_1 thallus phase. As *P. yezoensis* is monoecious, there is a possibility of self-fertilization occurring in the cross experiments together with cross-fertilization. Therefore, the conchocelis which segregates color types in F_1 thallus phase is assumed to be a cross-fertilized heterozygote; the conchocelis which produced only thalli of the maternal phenotype is assumed to be a self-fertilized homozygote. The heterozygous conchocelis formed in the reciprocal crosses were all the wild type, indicating that the mutants are recessive to the wild type and inherit in a Mendelian manner.

Though the heterozygous conchocelis segregated only the parental color types in the F_1 thallus, most of the thalli were sectorially variegated chimeral thalli in which a single thallus was zoned into different colors. Table 2 shows the color types and the frequencies of the chimeral thalli occurred from the heterozygous conchocelis. The frequencies of the chimeral thalli were 97.4%($W_{(♀)} \times R_{(♂)}$), 94.3%($R_{(♀)} \times W_{(♂)}$) and 92.9%($G_{(♀)} \times W_{(♂)}$) in respective cross. Observed chimeral thalli were as follows: two-sectorial chimera consisted of two different color types (Fig. 1); three-sectorial chimera in which one of the two color types was repeated (Fig. 2); and four-sectorial chimera in which both of the two color types were alternately repeated.

Crosses between the red type and the green type mutants

The heterozygous conchocelis formed in the reciprocal crosses were all the wild type, and segregated the red, the green, the wild and the yellow phenotypes in F_1 thallus phase (Table 3). The red type mutant and the green type mutant are assumed to complement each other because the heterozygous conchocelis is the wild type in spite of the fact that the mutants are recessive to the wild type. Thus, the yellow type and the wild type are regarded to be produced by a recombination of the loci of the red type and the green type mutants.

Chimeral thalli also appeared from these heterozygous conchocelis. The frequencies of the chimeral fronds were 99.5% when the red type was female and 97.5% when the green type was female (Table 4). Chimeral thalli produced from the conchocelis

Table 2. Color types and frequency of the thalli developing from conchospores released by the heterozygous conchocelis from the cross of the red and the wild types (I), and by the heterozygous conchocelis from the cross of the green and wild types (II) in *Porphyra yezoensis*. W, wild type; R, red type; and G, green type.

(I)

Color types	Number of F_1 thalli	
	W×R*	R×W*
Single color thalli		
W	26	11
R	24	18
Chimeral thalli		
W+R	1019	338
W+R+W	280	77
R+W+R	316	61
W+R+W+R	2	0
Frequency of chimeral thalli	W×R*	97.4%
	R×W*	94.3%

(II)

Color types	Number of F_1 thalli	
	G×W*	
Single color thalli		
W	186	
G	114	
Chimeral thalli		
W+G	2584	
W+G+W	691	
G+W+G	637	
W+G+W+G	16	
Frequency of chimeral thalli	G×W*	92.9%

* Female parent shown first.

Table 3. Results of the reciprocal crosses between the red and the green type mutants of *Porphyra yezoensis*. W, wild type; R, red type; G, green type; and Y, yellow type. (* Female parent shown first.)

Cross*	F_1 conchocelis	F_1 thalli	Putative cross combination
R × G	—R	R	R × R (self.)
	—W	W, R, G, Y (chimeral thalli)	R × G (cross.)
G × R	—G	G	G × G (self.)
	—W	W, R, G, Y (chimeral thalli)	G × R (cross.)

consisted of various combinations of the four color types. Observed combinations were as follows: the two-color type chimera composed of two sectors and two color types (Fig. 4); the repeated two-color type chimera consisting of three sectors and two color types, one of which was repeated (Fig. 5); the three-color type chimera made up of three sectors and three color types (Fig. 3); and four-color type chimera made up of four sectors and four color types (Fig. 6). The number of chimeral sectors does not exceed four. Though all possible combinations of the four colors were observed among the two-, three- and four-color type chimeras, only two combinations of the red and the green types, or the yellow and the wild types (R+G+R, G+R+G, Y+W+Y, W+Y+W) were observed in the repeated two-color type chimeras.

Each color sector of the chimeral thalli of *P. yezoensis* is regarded as a haploid phenotype which arose from meiotic segregation. If meiosis has been completed in the conchosporangium (MIGITA 1967, KITO 1978), the conchospore which produces a chimeral thallus should contain two to four haploid nuclei similar to the mosaic in *Gracilaria* (VAN DER MEER 1977). However, *P. yezoensis* produces only uninucleate conchospores (MIGITA 1967, KITO 1978). Provided that the chimeral thalli should be produced from the uninucleate conchospores, then we can assume that the conchospores are released during meiotic prophase to segregate haploid

Table 4. Color types of the thalli developing from the conchospores released by the heterozygous conchocelis from the cross of the red and the green types in *Porphyra yezoensis*. W, wild type; G, green type; and Y, yellow type.

Color types	Number of F_1 thalli R × G*	G × R*
Single color thalli		
R	3	44
G	1	20
Y	1	19
W	2	22
Chimeral thalli		
R+G	401	1032
W+Y	138	340
R+Y	38	130
R+W	32	90
G+Y	12	46
G+W	33	120
R+G+R	92	265
G+R+G	61	311
Y+W+Y	24	73
W+Y+W	15	71
R+G+Y	140	394
R+G+W	137	413
R+Y+W	134	410
G+Y+W	138	386
R+G+Y+W	0	2
Frequency of chimeral thalli	R × G*	99.5%
	G × R*	97.5%

* Female parent shown first.

genotypes during their germination, and this leads to the formation of variegated chimeral thalli.

The chimeral thalli of *P. yezoensis* are interesting because they are haploid but have various phenotypes. The mechanism of the formation of chimeral thalli is furthe to be clarified for genetic analysis of the mutants and also for the discussion of meiosis in *P. yezoensis*.

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大目 優・国藤恭正・三浦昭雄： スサビノリの色素変異体の交雑実験

スサビノリ (*Porphyra yezoensis* UEDA) の赤色型および緑色型変異体を用いて変異型と野生型および赤色型と緑色型との交雑実験を行った。その結果，変異型は野生型に対して劣性形質であることがわかった。また，赤色型と緑色型との交雑の結果生じた異型接合型糸状体は野生型を示した。このことは，赤色型と緑色型の遺伝子は相補的に作用した遺伝子座が異なることを示す。さらに次代葉状体期に赤色型と緑色型のほかに黄色型と野生型を分離した。このことは，遺伝子間の組み換えの結果，新しく黄色型と野生型が生じたことを示している。また，色彩型に関する異型接合型糸状体から生じた 92.9-99.5%の葉状体は区分状斑入りキメラ葉状体であった。これらのキメラ葉状体の高頻度の出現は，スサビノリでは減数分裂が殻胞子の発芽時に起ることを示唆している。
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