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Hybrid Breakdown and Breakthrough in Interspecific Crosses between *Porphyra yezoensis* and *P. tenera*

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Abstract Interspecific cross experiments between *Porphyra yezoensis* and *P. tenera* were performed using their color mutant types as genetic markers. In reciprocal crosses, most of the F_1 gametothalli developed from conchospores released from the wild-type F_1 sporothallus (conchocelis) became extinct at the four-cell stage with plastid collapse in the cells. A few uniformly pigmented gametothalli and sectorially variegated chimeric gametothalli survived. As meiosis of *P. yezoensis* and *P. tenera* is completed at four-cell stage of gametothallus (conchospore germling), the extinction of gametothallus at the four-cell stage is considered to be possibly due to some disorder at the meiosis. Therefore, the extinction of gametothallus is eqivalent to hybrid breakdown. This implies that the hybrid breakdown occurs not in sporothallic generation but in the gametothallic one in this interspecific cross. The survival gametothallus passing through the hybrid breakdown is equivalent to breakthrough. Breakthrough individuals are judged to be useful breeding material, and show that incomplete mechanism of reproductive isolation exists between *P. yezoensis* and *P. tenera*. This suggests the reality of biological species in genus *Porphyra*.

Key words: breakthrough, hybrid breakdown, interspecific cross, *Porphyra tenera*, *Porphyra yezoensis*.

Suto (1963) tried artificial cross experiments between 5 species including 4 local forms of genus *Porphyra*. Crosses easily occurred in any cross combination of *Porphyra* species and local forms tested. The descendants from intraspecific crosses grew normally, while the conchospore germlings (gametothalli) from interspecific crosses died at 2 to 5 cell stage with great frequency; only 2 to 20 percent of the germlings survived. The survivors either grew and matured normally or grew abnormaly in shape depending on cross combinations. The abnormal gametothalli were assumed to be "heteroploid" nature, having haploid chromosomes from the both parents together in their cells despite no cytological obvervation.

Miura (1985), Ohme *et al.* (1986), Ohme and Miura (1988), Niwa *et al.* (1993) and Miura and Ohme-Takagi (1994) performed interspecific cross experiments between wild-type *Porphyra yezoensis* and color mutants of red, green, yellow, violet, light red, light green and light yellow-types. They revealed that in *P. yezoensis* meiosis took place during conchospore germling stage, and thus the four-cell conchospore germling was equivalent to the ordered tetrad as in fungi *Neurospora*. Conchospores released from homozygous F_1 sporothalli (conchocelis) developed into uniformly pigmented gametothalli being the same as maternal color type. Those from monohybrid sporothalli could theoretically yield 6 types of sectorially variegated chimeric gametothalli and those from dihybrid, 36 types. By using these results, Miura (1985),

Table 1. Results of reciprocal crosses between the red-type (T-5) in *Porphyra yezoensis* and the green-type (T-8) in *P. tenera*. R, red-type; G, green-type; W, wild-type.

Cross*	F_1 sporothallus	F_1 gametothallus	Putative cross combination
R×G (T-5) (T-8)	R	R	(R×R)
	W	R, G, W, Chimeras, Extinct germlings	(R×G)
G×R (T-8) (T-5)	G	G	(G×G)
	W	G, R, W, Chimeras, Extinct germlings	(G×R)

*Female parent shown first.

Ohme and Miura (1988), Miura and Ohme-Takagi (1994) and Hamada *et al.* (1994) showed linkage maps.

We performed interspecific cross experiments between *Porphyra yezoensis* and *P. tenera* using their color mutants as genetic markers.

Materials and Methods

The gametothallus of the red-type mutant (strain: T-5) of *Porphyra yezoensis* and of the green-type (T-8) of *P. tenera* developed from conchospores released from their respective homozygous sporothalli were used in this study. The method of laboratory culture and artificial cross followed Ohme *et al.* (1986), Ohme and Miura (1988), Miura *et al.* (1992) and Miura and Ohme-Takagi (1994).

Results and Discussion

Table 1 shows the inheritance mode of color types in the reciprocal crosses between the red-type mutant (T-5) of *Porphyra yezoensis* and the green-type (T-8) of *P. tenera*.

When the red-type mutant (T-5) of *P. yezoensis* was female and the green-type (T-8) of *P. tenera* male, both the red-type and the wild-type F_1 sporothalli occurred. All of the F_1 gametothalli developed from conchospores released from the red-type F_1 sporothallus were red-type, and grew normally. Most of the F_1 gametothalli developed from the conchospores released from the wild-type F_1 sporothallus became extinct at the four-cell stage with plastid collapse in cells. Only a few uniformly pigmented gametothalli of the red-type, green-type, wild-type, as well as a few sectorially variegated chimeric gametothalli composed of those color types survived. Fig. 1 shows the developmental course and collapsing progress of plastids in cells of the gametothallus developed from the specified conchospore released from the heterozygous F_1 sporothallus in the cross of the red-type (T-5) of *P. yezoensis* with the green-type (T-8) of *P. tenera*. The plastid in the cell of gametothallus collapsed successively from the upper to the basal cell when the gametothallus grew into the four-cell stage.

In the reciprocal cross, when the green-type mutant (T-8) of *P. tenera* was female and the red-type (T-5) of *P. yezoensis* male, both the green-type and the wild-type F_1 sporothalli occurred. All of the F_1 gametothalli developed from conchospores released from the green-type F_1 sporothallus were green-type, and grew normally. Most of the F_1 gametothalli developed from conchospores releaesed from the wild-type F_1 sporothallus became extinct at the four-cell stage with plastid collapse in cells. Only a few uniformly pigmented gametothalli of the green-type, red-type wild-type, as well as a few sectorially variegated chimeric gametothalli com-

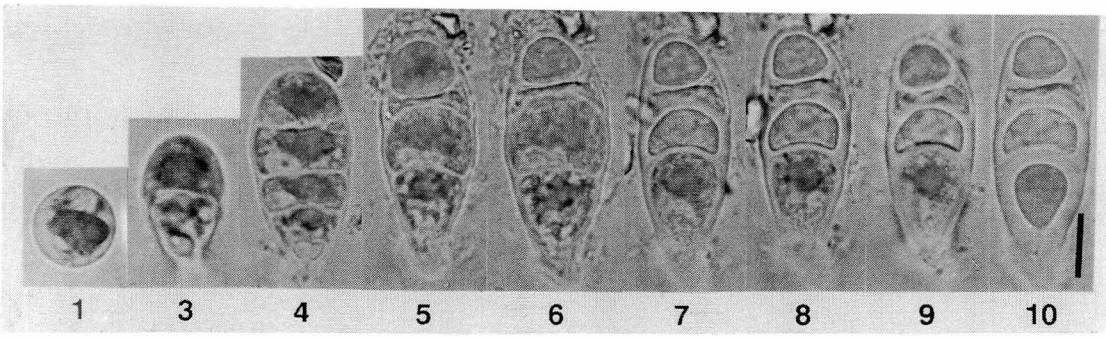

Fig. 1. Photomicrographs showing the developmental course of the gametothallus developed from the specified conchospores released from the heterozygous F_1 sporothallus in the cross between the red-type (T-5) in *Porphyra yezoensis* and the green-type (T-8) in *P. tenera*. Numerals denote days following conchospore release. Note the plastid in the cell collapsing successively from the upper to the basal cell at four-cell stage after the fifth day. Scale bar = 10 μm.

posed of those color types survived.

In the present reciprocal crosses, all of the red-type and the green-type F_1 gametothalli produced from theer respective parental types continued to live. The red-type and the green-type F_1 sporothalli proved themselves as self-fertilized homozygotes like the case of intraspecific crosses in *P. yezoensis* (Miura, 1985; Ohme *et al.*, 1986; Ohme and Miura, 1988; Miura *et al.*, 1992; Niwa *et al.*, 1993; Miura and Ohme-Takagi, 1994). Since the wild-type F_1 sporothalli segregated into color types in the F_1 gametothalli, they proved themselves as cross-fertilized heterozygotes like the case of intraspecific crosses in *P. yezoensis* (Miura, 1985; Ohme *et al.*, 1986; Ohme and Miura, 1988; Miura *et al.*, 1992; Niwa *et al.*, 1993; Miura and Ohme-Takagi, 1994). Simultaneous occurrence of both the homozygote and the heterozygote in F_1 sporothalli occur because *P. yezoensis* and *P. tenera* are both monoecious, making both self- and cross-fertilization possible. As mentioned above, inheritance mode of color types in interspecific cross between *P. yezoensis* and *P. tenera* is the same as that of intraspecific cross between *P. yezoensis*. Interspecific crosses between *P. yezoensis* and *P. tenera* greatly differ from intraspecific crosses between *P. yezoensis* in that it has a very high frequency of extinction of individuals in four-cell stage of gametothallus.

The yellow type gametothallus appeared from the intraspecific cross between the red-type and the green-type mutants in *P. yezoensis* did not appear in this interspecific cross; the reason remains unknown.

Table 2. Frequency of color types in the gametothalli resulting from conchospores rereased from the heterozygous sporothalli in crosses between the red-type (T-5) in *Porphyra yezoensis* and the greentype (T-8) in *P. tenera*. R, red-type; G, green-type; W, wild-type.

Color type	Number of thalli	
	R×G*	G×R*
Single-colored thalli		
R	15	140
G	122	6
W	11	4
Chimeric thalli		
R+W	2	3
G+W	1	—
G+R+G	—	1
Extinct germlings	1675	1521
Frequency of chimeric thalli	0.16%	0.24%
Frequency of extinct germlings	91.7%	90.8%

*Female parent shown first.

Table 2 shows color types in the F_1 gametothalli resulting from conchospores released from the heterozygous F_1 sporothalli in interspecific reciprocal crosses between the red-type (T-5) of *P. yezoensis* and the green-type (T-8) of *P. tenera*.

In the interspecific crosses, F_1 gametothalli ceased cell division at their four-cell stages, becoming extinct individuals with a high fre-

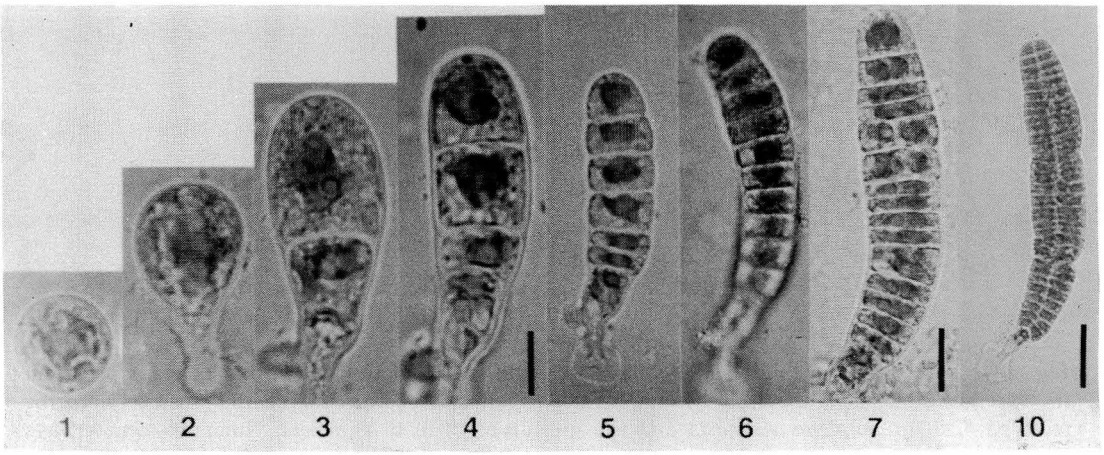

Fig. 2. Photomicrographs showing the developmental course of the single-colored gametothallus survived among the gametothallic germlings most of which were extinct at the four-cell stage. This gametothallus developed from the specified conchospore released from the heterozygous F_1 sporothallus in the cross between the red-type (T-5) in *Porphyra yezoensis* and the green-type (T-8) in *P. tenera*. Numerals denote days following conchospore release. This gametothallic germling is a breakthrough. Scale bar=10 μm in 1 to 4; 20 μm in 5 to 7; 50 μ m in 10.

quence of collapsing plastids. The frequency of extinction of gametothalli was 91.7% in the cross between the red-type (T-5) of *P. yezoensis* and the green-type (T-8) of *P. tenera*, and 90.8% in T-8×T-5. The appearance frequency of sectorially variegated chimeric gametothalli was 0.16% in T-5×T-8 and 0.24% in T-8×T-5. These frequencies are extremely low compared with those frequencies reaching upward of 90% at all times in intraspecific reciprocal crosses between the red-type and the green-type of *P. yezoensis* (Ohme *et al.*,1986). Only 3 of a possible 36 types of sectorially variegated chimeric gametothalli were observed in these interspecific crosses. The survivors, not exceeding 10%, grew and formed reproductive cells. Figure 2 shows the developmental course of the uniformly pigmented gametothallus of wild-type survived among the gametothalli most of which became extinct at four-cell stage.

The results show that the event of extinction at four-cell stage of F_1 gametothalli produced from heterozygous F_1 sporothalli in interspecific crosses between *P. yezoensis* and *P. tenera* is equivalent to hybrid breakdown (Dobzhansky, 1970; White, 1978; Grant, 1981). Meiosis of *P. yezoensis* and *P. tenera* is completed at the four-cell conchospore germling stage (gametothallic phase) (Ma and Miura, 1984; Ohme *et al*, 1986; Ohme and Miura, 1988; Tseng and Sun, 1989; Miura and Ohme-Takagi, 1994). In the interspecific crosses between *P. yezoensis* and *P. tenera*, hybrid breakdown occurs not in the sporothallic (haploid) phase but in the gametothallic (diploid) phase. Thus, the hybrid breakdown in gametothallus is considered to bedue to some disorder at the meiotic stage (van der Meer, 1987; Cole, 1990); consequently, the survival gametothallus passed through the hybrid breakdown is equivalent to the breakthrough (Hadorn, 1945). Breakthrough is judged to be a useful genetic variation in breeding material (Shin and Miura, 1995; Shin *et al.*, 1996). Furthermore, hybrid breakdown suggests that incomplete mechanism of reproductive isolation exists between *P. yezoensis* and *P. tenera*, which is the reality of biological species (Mayr, 1966, 1982, 1988; Mayr and Ashlock, 1991) in genus *Porphyra* as well as other red algae (Guiry, 1992).

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スサビノリとアサクサノリとの種間交雑における雑種崩壊とブレイクスルー

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スサビノリの赤色型（系統：T-5）とアサクサノリの緑色型（系統：T-8）を交雑親として種間交雑実験を行った．正逆交雑の結果，野生型の F_1 異型接合型糸状体から放出された殻胞子が発芽した F_1 葉状体の大部分は4細胞期で色素体が崩壊して死滅し，少数の一色彩型葉状体とこれらの色彩型区分からなる区分状斑入りキメラ葉状体が生き残った．スサビノリとアサクサ

ノリの減数分裂は殻胞子発芽体（葉状体）の4細胞期で完了するので，4細胞期のF_1葉状体が死滅するのは減数分裂の過程で何らかの異常が起こることに起因するものであると考えられる．したがって，この種間交雑における4細胞期のF_1葉状体の死滅は雑種崩壊に相当し，この雑種崩壊は造胞世代でなく配偶世代で起こる．雑種崩壊のときの生き残り個体はブレイクスルー(breakthrough)に相当し，このブレイクスルーは育種素材として有用である．また，雑種崩壊の現象とブレイクスルーの現出は，スサビノリとアサクサノリの両種間には不完全な生殖的隔離機構が存在し，アマノリ属における生物学的種の実態を示唆している．