A Life History and Hybridization of *Antithamnion sparsum* Tokida (Rhodophyta, Ceramiaceae) in Culture

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紅藻 *Antithamnion sparsum* Tokida 의 生活史 및 交配에 關한 研究

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ABSTRACT

Antithamnion sparsum Tokida isolated from the southern and western coasts of Korea was investigated in culture, comparing the morphological character with *A. defectum* Kylin from the Pacific North America. *A. sparsum* basically showed a *Polysiphonia*-type life history. However, it sometimes exhibited a monoecious reproduction and the carpospores released from the cystocarp by self-fertilization unexpectedly developed into the plants bearing spermatangia alone. These male plants were not functional up to 60 days culture. The results of intraspecific crosses between populations of *A. sparsum* were positive and the hybrid carpospores gave rise to normal tetrasporophytes.

On the other hand, the interspecific crosses between *A. sparsum* and *A. defectum* were successful partly, evidenced by the gonimoblast development and the release of carpospores in case of *A. sparsum* (male) x *A. defectum* (female), but not in case of *A. sparsum* (female) x *A. defectum* (male). Thus, the both species were still under the speciation.

INTRODUCTION

Several species of *Antithamnion* have been cultured in laboratory. They are considered to have a regular sequence of life history with tetrasporophyte, dioecious gametophyte, and carposporophyte (Drew, 1955; Sundene, 1959; Lee and West, 1980). However, some of them show irregular reproductive cycles in addition to a typical *Polysiphonia*-type of life history (Sundene, 1964; West and Norris, 1966; Rueness and Rueness, 1973), whereas some others repeat the tetrasporic generations (Sundene, 1962) or vegetative growth alone in laboratory culture (Whittick and Hooper, 1976).

The life history of *A. sparsum* has not been confirmed in laboratory culture. They are

expected to show a typical *Polysiphonia*-type life history, even though the cystocarpic plants are not reported in the field (Tokida, 1932, 1954; Kang, 1966; Noda, 1970; Lee and Kim, 1977). The plants distributed from Saghalien to Korea (Tokida, 1932; Kang, 1966), and are closely related to *A. defectum* occurring to the Pacific North America (Tokida, 1932). The both have been distinguished by the cell dimension, position of tetrasporangia, and gross morphology (Tokida, 1932), which however can be subject to the environmental influence (Sundene, 1962). Thus, Wollaston (1971) suggests that *A. sparsum* may be conspecific with *A. defectum*, and Yoshida (1981) recently proposes the former is placed as a synonym of the latter.

In this paper the life history and reproduction of *A. sparsum* from Korea are investigated in laboratory culture, and the assessment of the species is considered by interspecific cross with *A. defectum* from the Pacific North America.

MATERIALS AND METHODS

Two isolates of *A. sparsum* were used for this study. One (#138) from the southern coast of Korea was obtained at the intertidal zone of Jamdo, Jinhae Bay (35°03′N, 128° 40′E) on November 17, 1979, and the other (#238) from the western coast of Korea was at Gopado, Garolim Bay (36°24 ′N, 126°21 ′E) on May 15, 1980. They were placed in a cooler and transferred to the laboratory for culture. For interspecific cross experiments, on the other hand, culture strain of *A. defectum* (JAW #240, 241: Lee and West, 1980) from Californian coast was obtained by air shipment with the courtesy of Dr. West, UC Berkeley on October 8, 1980.

Method for isolation into unialgal culture was followed by Lee and West (1980). Preculture of all isolates was maintained in 1/2 PES medium under cool white fluorescence light below 300 lux. After 3~7 days, they were transferred to the incubation condition; in full strength PES media, under 16~19°C, 800~1300 lux, 16:8 LD, using $7 \times 7 \, cm^2$ glasswares. In order to eliminate diatoms GeO_2 solution was added to the culture medium for a while (West, 1970). The medium was usually changed in every forthnight.

Tetraspores were obtained from full mature tetrasporangia. After 24 hours, the sporelings were transferred to culture dishes. Carpospores were cultured in a same manner as the tetraspores. In order to observe the fertilization, the plants bearing cystocarps were isolated individually and cultured for a while to make sure that unfertilized young branches were grown newly. In addition, a few excised apices of a female plant were kept singly in a glassware for observation of a possible parthenogenesis.

RESULTS

The vegetative development of all isolates of *A. sparsum* is identical in the laboratory culture. There is also no detectable difference in vegetative morphology of tetrasporo-

phyte to those plants described from the field (Tokida, 1932; 1954).

Vegetative Morphology. Erect thallus with prostrate base is 3(—5) cm high and attached to the glassware by means of rhizoidal filaments arising from spherical basal cells of the determinate branchlets. The rhizoidal filaments with blunt tips are 4~8 celled. They also arise on the upper portion of the thallus. The cells of main axis are 60 μm broad and 350 μm long, about six times as long as broad at maximum, which are contrast to 2~5.5 times in field (Tokida, 1932). Determinate branchlets on the main axis are opposite, usually 12~16 celled and semipinnately pectinate on upperside. Indeterminate branches arise from every 3(—7) segments of main branch and basically produce no branchlets at the opposite side.

Adventitious indeterminate branches sometimes arise from the basal cell of determinate branchlets. Hairs, not recorded in the field, occur frequently on the terminal cell of determinate branchlets in apical portion of the thallus associated with sexual reproductive structures. Gland cells usually locate on 2~3 cells of a pinnule. They are commonly 24 μm long and 19 μm broad.

Reproduction in Culture. Both tetraspores and carpospores are isolated from field for laboratory culture. Germination pattern is basically identical in the both spores. After attachment, the spores synchronously develop two opposite primordia, one forms a rhizoid and the other develops into an apical cell from which the erect frond appears (Figs. 1~5). Some sporelings, however, develop the rhizoidal cell much later, and the others the erect frond much later. Determinate branchlets at first arise alternately (Figs. 6 and 8), or sometimes secundly (Fig. 7) within 16~18 days after germination. Then, they become opposite after full growth.

The tetraspores grow into the gametophytes in 20~30 days after germination. Spermatangial ramuli are observed early, and carposporangial plants appear about 10 days later. Gametophytes are basically dioecious in culture. Spermatangia develop on all parts of the pinnules in determinate branchlets. Each cell of a spermatangial ramulus cuts off a few spermatangial parent cells, which divide once or a few times, forming 2 to 4 spermatia (Fig. 9).

Carpogonial branches are common in upper to apical portion of the thallus, occurring singly or very rarely in pairs successively on the basal cell of determinate branchlets along the main axis and laterals. The small basal cell bearing the carpogonial branch grows larger than others and becomes the supporting cell. A mature carpogonial branch develops a long trichogyne (Figs. 11 and 17).

It is known that a single carpogonial branch on each fertile apex usually matures into the cystocarp and the rest carpogonial branches are degenerated (Wollaston, 1968). However, during the culture two to three carpogonial branches not rarely fertilized and grow into mature cystocarps at the same time. Such an occurrence is not observed previously among the species of *Antithamnion*.

The development of the carposporophyte is basically similar to those by Wollaston
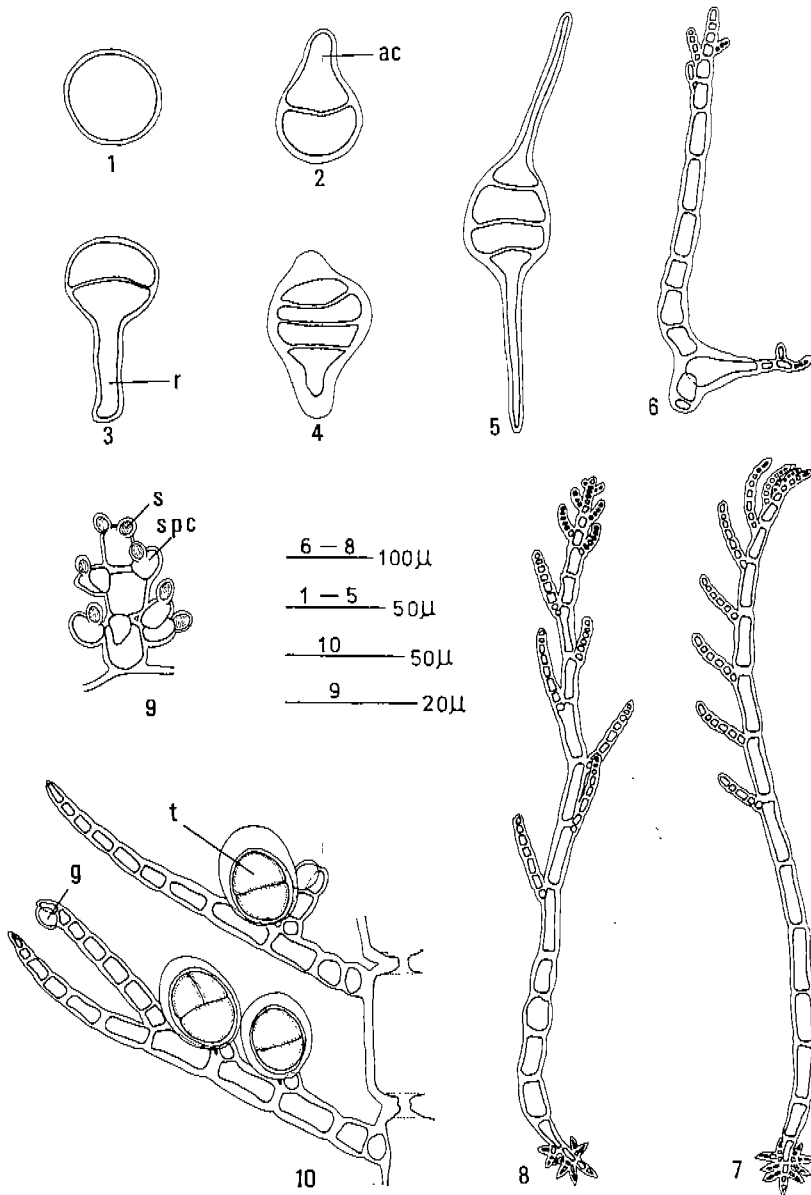
Figs. 1~10. Developments of vegetative thallus, tetrasporangia and spermatangia of *Antitham-nion sparsum* in culture.

Fig. 1. Released tetraspore. Figs. 2~3. One-day tetrasporelings. Figs. 4~5. 4-celled stage with bipolar apices. Figs. 6~8. Branching types of young plant. Fig. 9. Development of spermatangia. Fig. 10. Development of tetrasporangia (ac: apical cell, g: gland cell, r: rhizoidal cell, s: spermatangial parent cell, t: tetrasporangium).
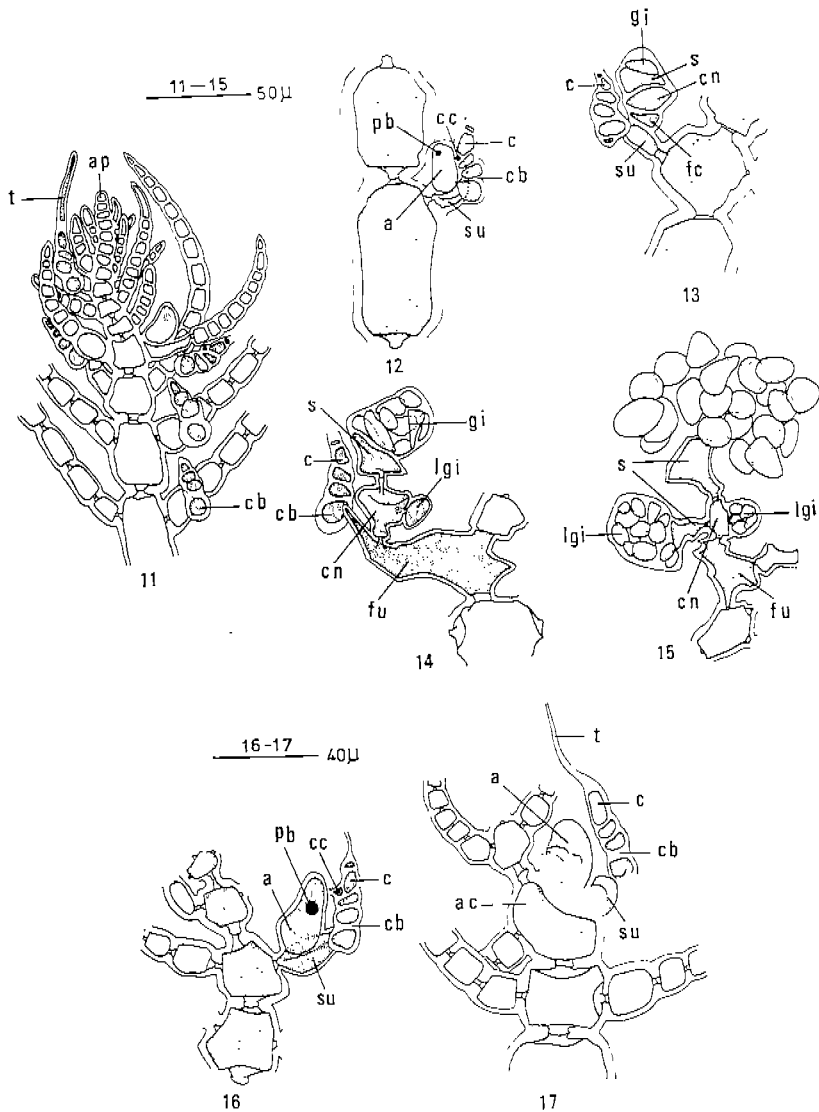
Figs. 11~15. Development of female reproductive structure of *Antithamnion sparsum* Tokida in culture.

Fig. 11. Procarps in apical portion of main axis. Fig. 12. Auxiliary cell and connecting cell. Fig. 13. Development of early gonimoblast cells. Fig. 14. A young cystocarp with secondary gonimoblast initial. Fig. 15. A mature cystocarp (a: auxiliary cell, ap: branch apex, c: carpogonium, cc: connecting cell, cn: central cell, fc: foot cell, fu: fusion cell, gi: gonimoblast initial, pb: protein body, s: sterile cell, su: supporting cell, t: trichogyne).

Figs. 16~17. Unsuccessful development of gonimoblast in interspecific cross of *Antithamnion sparsum* (female) and *A. defectum* (male) in culture.

Fig. 16. Development of connecting cell after fertilization. Fig. 17. Stop to develop early gonimoblast cell (a: auxiliary cell, ac: axial cell, c: carpogonial branch, cc: connecting cell, pb: protein body, su: supporting cell, t: trichogyne).

(1968) and Lee and West(1980). The enlarged supporting cell, after fertilization, cuts off a characteristic dome-shaped auxiliary cell and becomes acetabuliform. The carpogonium, cutting off the trichogyne and leaving a cap cell at the top, produces a connecting cell that fuses to the auxiliary cell (Fig. 12; Wollaston, 1968; Lee and West, 1980). The presumed diploid nucleus is removed into the auxiliary cell via this connecting cell. After fusion with the connecting cell, the auxiliary cell divides transversely to form the lower foot cell and upper central cell, which gives rise to the gonimoblast initials (Fig. 13). The gonimoblast cell is produced terminally on the auxiliary cell and at this stage a strong fusion occurs among the axial cell, supporting cell and the foot cell (Fig. 14). No special involucre is formed but the pinnae of axial cells below the cystocarp grow upwards, surrounding the mature cystocarp partially (Fig. 15). The carpospores are released in 30 days since the formation of auxiliary cell.

Carpospores in 30 days after germination grow to tetrasporophytes that produce tetrasporangia. Mature tetrasporangia are ovoid to ellipsoidal and 41×59 μm on an average. Cruciately divided tetrasporangia are pedicellate in one or two cells, or sessile on the upper part of the pinnae of determinate branchlets in contrast to the description by Tokida (1954), who mentioned they were one pedicellate or sessile (Fig. 10). Tetrasporangia release tetraspores in two weeks after appearance.

Thus, about 4 months are required in culture to complete the full cycle of the plant. A typical *Polysiphonia*-type life history is repeated for three times during the culture.

Unusual Life Histories. On the other hand, several monoecious gametophytes (♯138～ 522) derived from male gametophytes are observed in Jamdo isolates during the culture. Each monoecious gametophyte, isolated individually, develops cystocarps, indicating self-fertilization. However, all the carpospores released germinate and unexpectedly grow into the plants bearing spermatangia. The morphological characteristic of the plants bearing spermatangia is quite similar to a common male gametophyte. The fertility of these spermatia is not confirmed confidently. Even though these carpospore-originated spermatangial plants are put together with normal female plants for 60 days, no cystocarps are developed.

Table. 1. Cross experiments among populations of *Antithamnion sparsum* from Korea and *A. defectum* from Pacific North America

Female			Male		Fertilization	Carpospore release
A. sparsum	♯138	x	*A. sparsum*	♯138	+	+
A. defectum	♯240	x	*A. defectum*	♯241*	+	+
A. sparsum	♯138	x	*A. sparsum*	♯238	+	+
A. defectum	♯240	x	*A. sparsum*	♯138	+	+
A. sparsum	♯138	x	*A. defectum*	♯241	+	—

*Lee and West (1980)

Intra- and Inter-Specific Crosses. The cross experiment between Jamdo isolate (♯138) and Garolim Bay isolate (♯238) of *A. sparsum* shows a positive result, producing viable carpospores. In addition, all crosses between male *A. sparsum* and female *A. defectum* are also successful by the fact that they develop normal cystocarps and viable carpospores (Table 1). However, as seen in the Table 1, the crosses between female *A. sparsum* and male *A. defectum* produce no mature cystocarp nor viable carpospores. The gonimoblast stops grow during the early development (Figs. 16 and 17).

DISCUSSION

As summarized in Figure 18, *Antithamnion sparsum* basically shows a typical *Polysiphonia*-type of life history (Drew, 1955; Sundene 1959; Lee and West, 1980). Some unusual appearance of reproductive structures is also reported among the species of *Antithamnion* not only in culture (Sundene, 1962, 1964; West and Norris, 1966; Rueness and Rueness, 1973), but also in the field (L'Hardy-Halos, 1968; Knaggs, 1969).

However, *A. sparsum* shows another unusual phenomenon in life history by the monoecious reproduction, such as the female reproductive structures are developed on the male gametophyte, and the resulting carpospores, missing the tetrasporic phase, develop exclusively male gametophytes of which spermatia however are not functional. On the contrary no female plant develops monoecious male branches in the present culture.

There are two reports on the monoecism of *Antithamnion* in culture (Drew, 1955; West and Norris, 1966). Thus, the monoecism may occur not rarely in this genus as it is apparently common in *Callithamnion*, a related genus. However, it is peculiar that such monoecious plants miss the tetrasporophyte as seen in the present experiment. Whittick and West (1979) demostrated the life history of a monoecious species of *Callithamnion* and found that the carpospores from the cystocarp indicating self-fertility develop into the tetrasporophytes as seen in regular dioecious plants. Polanshek and West (1977) reported the repeating of cystocarpic generations in the life history of *Gigartina papillata*. However, the lack of tetrasporic generation in *A. sparsum* is not equivalent to these results.

Sundene (1962, 1964) and West and Norris (1966) reported the apomeiotic tetraspores on the gametophyte of *Antithamnion* developed into the same gametophyte as the parent in sexuality, whereas Rueness and Rueness (1973) reported that the tetraspores on the male gametophytes developed into the male and female plants, too. Such a phenomenon is also observed in *Dasysiphonia chejuensis* (Lee and West, unpublished data). Rueness and Rueness (1973) mentioned that the light condition seemed to role an important factor to induce the sexual reproductive structures.

Van der Meer and Todd (1977) reported mixed phase reproduction in the life history of *Gracilaria* sp., and suggested that the sexuality was controlled by the genetic recom-

Table 2. A comparison of some significant taxonomic characters between *Antithamnion sparsum* sensu Tokida and *A. defectum* Kylin

Characters	A. defectum	A. sparsum	A. sparsum
Attachment	rhizoidal	rhizoidal	rhizoidal
Branching pattern	opposite	opposite	opposite
Cell dimension	2-3 times	2-5.5 times	5-6 times at maximum
Cell tip	tapering	blunt	blunt & tapering
Gland cell	on 2-5 cells	on 2-3 cells	on 2-3 cells
Tetraporangia	1-2 pedicellate ovoid/80 μm long	1-pedicellate sessile/ovoid 59×78 μm	1-2 pedicellate sessile/ovoid 41×59 μm
Spermatangia	adaxial	adaxial	adaxial
References	Kylin(1925) Wollaston (1971)	Tokida(1932, '54) Lee and Kim(1977)	In this paper

bination of a pair of alleles rather than a pair of chromosomes. But this was in case of diploid tetrasporophytes. He did not explain the mixed reproduction in the gameto-phyte observed by West and Norris (1966), and Rueness and Rueness (1973).

The monoecism of *A. sparsum* seems to be genetically stable by the fact that once the female branches are developed on the male thallus, they never return to the male, and produce cystocarps successively, as in cases of tetrasporangium formation on cult-ured gametophytes of *Symphyocladia pennata* and *Dasysiphonia chejuensis* (Lee and West, 1979). The present peculiar monoecism may be clarified partially by the cytological investigation of the plant during the unusual life history. However, the occurrence of nonfunctional spermatia from such male thalli predicts that these gametophytes would be diploid in contrast to common haploid ones.

Reporting *A. sparsum* as a new species, Tokida (1932) mentioned this species showed more affinity to *A. defectum* Kylin, and the both basically were distinguished by the difference of cell dimension. Wollaston (1971) doubted therefore the both species might be conspecific, and Yoshida (1981) treated the former as a synonym of the latter. In fact, such morphological characters adopted for distinguishment of the species are easily subject to the environmental influence (Sundene, 1962; Norris and West, 1967), or to be vague (Wollaston, 1968), and also demonstrated to be of little value by this study. A comparison of some significant taxonomic characters between *A. sparsum* sensu Tok-ida and *A. defectum* Kylin is shown in Table 2.

According to the interspecific cross experiment, the both species are partially inter-fertile by the formation of carposporophyte and production of viable carpospores in case of *A. sparsum*(male) × *A. defectum*(female). However, the reciprocal cross between the female *A. sparsum* and the male *A. defectum* is not successful. They produced auxiliary cell after fertilization, but fail to develop gonimoblast cells (Figs. 16 and 17), which suggest the both species are still under the speciation.
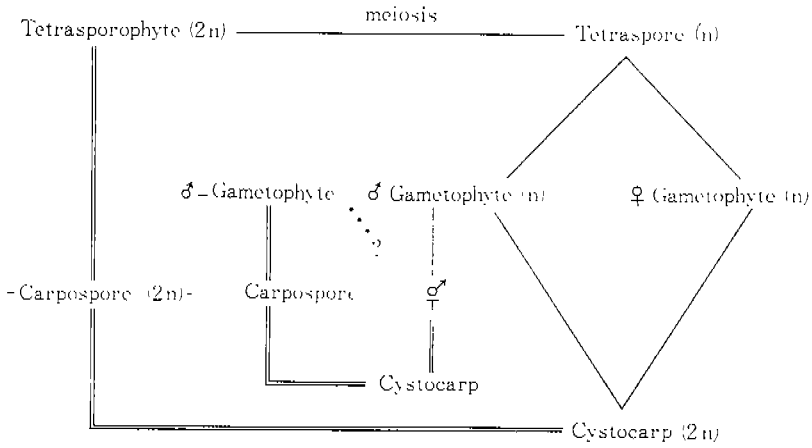
Tetrasporophyte (2n) ————————— meiosis ————————— Tetraspore (n)

♂ – Gametophyte   ♂ Gametophyte (n)   ♀ Gametophyte (n)

- Carpospore (2n) -   Carpospore   ♂

Cystocarp

Cystocarp (2n)

Fig. 18. A life history of *Antithamnion sparsum* Tokida in culture.

摘   要

홍조 웃가지 안티밤니온(*Antithamnion sparsum* Tokida)을 서해 가로림만과 남해 잠도에서 채집하여 실내배양으로 그 생활사를 조사하였고, 북미 대평양산 *A. defectum*과 교배실험을 수행하였다. 본 식물은 홍조류의 전형적인 폰리시포니아형 생활사를 보여주지만, 배양중 자웅동체인 개세가 나타나서 이를 분리 배양한 결과, 기본 생활사와는 달리 수정 후 과포자를 형성한 다음 이들이 모두 웅성체로 되었다. 또한 한 개의 fertile branch에서 2~3개의 태원열이 동시에 수정하여 성숙하는 특이한 현상도 관찰되었다. 한편, 본종의 서해집단과 남해집단간의 교배는 정상적인 수정과정을 거쳐서 과포자를 형성하였다. 그러나, 한국산 *A. sparsum* 웅성체와 북미산 *A. defectum* 자성체 사이에서는 정상적인 교배가 이루어져서 수정을 통한 과포사 형성이 이루어졌지만, 한국산 자성체와 북미산 웅성체 사이에는 수정은 하였으나 과포자 형성 과정에서 생식세포군이 죽어버리는 블렬싱을 나타내있다. 따라서, 이 두 종은 현게 종분화가 이루어지고 있는 과정에 있는 종류들임을 알 수 있었다.

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