INTERGENERIC AND INTERSPECIFIC CROSSINGS
OF THE LAVERS (PORPHYRA)*

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Introduction

Recent advancements in cultural techniques for the seaweeds, for instance, Porphyra and Undaria, through various stages of their development have brought about remarkable benefit to a substantial portion of local fishermen engaged in this type of work in Japan.

However, little attention has been drawn to possibility for artificial crossing of seaweeds that may produce a species with better characters than those available at present for the harvest as rich as can be expected. FøyN1,2) was the only worker that pursued in his laboratory subsequent generations he obtained by crossings between three forms of Ulva mutabilis. Sundene3,4) found that three forms in Laminaria digitata are interfertil. He succeeded in bringing up the hybrids to a normal size by placing them in the sea. Some authors found possibility of crossing of other seaweeds, but they failed in bringing up the following mature thalli most probably because of difficulties in the cultural techniques for these particular species.

The author5) succeeded in a laboratory culture of lavers, in which they grew normally and almost at the same growth rate as they do in nature. Thereupon, since 1958 he has been undertaking at his laboratory experiments on artificial crossings between several species and their local forms of commercially cultured lavers, with a view to obtaining new lavers which may have convenient characters for the culture. Recently the experiments succeeded in producing descendants through two or three generations*** and revealed that some of them grew more vigorously than their native parent plants did.

In a preliminary phase of the work prior to the crossing experiments efforts have been paid to clarifying a few questions hitherto held in regard to presence or absence of the fertilization and subsequent development as will be discussed later. Because there have been no evidences to prove or disprove a commonly accepted inference that a carpogonium, female cell of sexually mature Porphyra, is fertilized by a spermatium, male cell, and develops into a cystocarp, group of carpospores, which later germinate into filamentous bodies, Conchocelis.

In the present paper, which describes an account of these experiments, technical

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*** One generation is composed of a Conchocelis phase and a subsequent thallus phase.
terms commonly used for diploid plants are often avoided, because it is nearly consensus among algologists that thalli of *Porphyra* are haploid.

**Materials**

The following list shows species and local forms used in the experiments, their sexuality and habitats with abbreviations by which they will be presented in Tables 1 to 3.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sexuality</th>
<th>Locality sampled</th>
<th>Abb.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Porphyra pseudolinearis</em></td>
<td>Dioecious</td>
<td>Kesennuma Bay, Miyagi Prefecture</td>
<td>P</td>
</tr>
<tr>
<td><em>P. umbilicalis</em>?</td>
<td>*</td>
<td>Tokyo Bay</td>
<td>U</td>
</tr>
<tr>
<td><em>P. angusta</em></td>
<td>*</td>
<td>*</td>
<td>A</td>
</tr>
<tr>
<td><em>P. tenera</em></td>
<td>Monoecious</td>
<td>*</td>
<td>T</td>
</tr>
<tr>
<td><em>P. tenera</em>?</td>
<td>*</td>
<td>Matsukawa Inlet, Fukushima Pref.</td>
<td>Tk</td>
</tr>
<tr>
<td><em>P. tenera</em>?</td>
<td>*</td>
<td>Mangoku Inlet, Miyagi Pref.</td>
<td>Tg</td>
</tr>
<tr>
<td><em>P. tenera</em>?</td>
<td>*</td>
<td>Kesennuma Bay, Miyagi Pref.</td>
<td>Tp</td>
</tr>
<tr>
<td><em>P. yezoensis</em></td>
<td>*</td>
<td>Tokyo Bay</td>
<td>Y</td>
</tr>
</tbody>
</table>

The artificial fertilization was conducted by the use of carpospores of typical individual plants selected among samples of these species, and two or more generations were consecutively brought up. Thus, for subsequent crossing experiments, uniformity of the materials was morphologically confirmed but not genetically because of a limit of available facilities.

**Methods**

Immature thalli of the layers of 30 to 40 days old after the germination were set into individual culture. In 10 to 20 days later formations of carpogonia and spermatia were observed. Then, a mature part of a thallus with carpogonia was cut into four to five pieces. They were separately put into a series of culture vessels, each either in the presence or in the absence of some pieces of mature male thalli of anyone of the species or its local form under study. Two to four days later, the male pieces were taken out to follow the subsequent growth of the carpogonia. When carpospores were found developing from them, usually one week later, they were further cultured until more than two subsequent generations were brought up.

**Results**

1. **Growth of Fertilized or Unfertilized Carpogonia**

In order to ascertain probability of fertilization and ensuing developments of the layers under report, growth of the first two series of carpogonia were observed, one
made, and the other not made, contact with spermatia of the same species or its local forms. Reference to Table 1 showing the developmental results of these series reveals the following.

**Table 1. Development of carpogonia upon making or not making contact with spermatia of the same species.**

<table>
<thead>
<tr>
<th>State of development</th>
<th>Dioecious</th>
<th>Monoecious</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>U</td>
</tr>
<tr>
<td>No development</td>
<td>0(15)</td>
<td>0(1)</td>
</tr>
<tr>
<td>Leafy growth but no Conchocelis</td>
<td>0(2)</td>
<td>0(7)</td>
</tr>
<tr>
<td>Several Conchocelis</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Many Conchocelis</td>
<td>17(0)</td>
<td>8(0)</td>
</tr>
</tbody>
</table>

The numerals are the number of experiments in which carpogonia developed with receiving the spermatia. Ones in parentheses are those without spermatia.

See the material list for abbreviations of species names used in Tables 1 to 3.

i) **Dioecious layers**: Without receiving spermatia, carpogonia of the three dioecious species studied became decay, except a few that developed into groups of carposporale-like cells and germinated into leafy thalli. No *Conchocelis* was found developing. Carpogonia, which had a chance to contact with spermatia, developed into normal cystocarps, cells of which, i.e., carpospores, germinated into *Conchocelis*.

It can be concluded with the least reservation that the fertilization of dioecious layers is followed by carpospore formation and *Conchocelis* development, while unfertilized carpogonia, though they sometimes germinate into laver thalli, cannot develop into *Conchocelis*.

In the present work the fertilization was confirmed by observing the *Conchocelis* development rather than the cystocarp formation, in order to avoid misjudgement of false cystocarp of unfertilized cells for a true cystocarp, even though the latter method is theoretically right for the purpose as well.

ii) **Monoecious layers**: In monoecious species every thallus piece that has carpogonia also has spermatia, which inevitably have a chance to make contact with carpogonia. The fact would make it impossible to prevent carpogonia from being naturally fertilized. In the present work, a number of carpogonia, without receiving spermatia from other thalli, were found developing to carpospores, which grew into *Conchocelis*. It can be tentatively assumed, therefore, that the carpogonia could be fertilized by spermatia from the same individual plant. Fortunately, however, the self-fertilization rate was generally poor, sometimes negligible as compared with cross-fertilization. This enabled the author to carry out subsequent crossing experiments. In monoecious layers, leafy thalli were also found developing from the carpogonia as well and that the more frequently when the self-fertilization rate became the poorer. Therefore, the leafy
thalli may be supposed to develop from unfertilized carpogonia.

When carpogonia had contact with spermatia from another thallus of the same species, they usually developed into cystocarps, and later into *Conchocelis*. Therefore, the same conclusions as drawn upon the dioecious layers may also hold valid with the monoecious layers: 1) the occurrence of fertilization, 2) the following development to cystocarps, carpospores and *Conchocelis* and 3) practicability of confirming the fertilization according to *Conchocelis* development—though the self-fertilization may somewhat confuse the situation.

2. Crossing Experiments

Table 2 indicates the growth of carpogonia apparently fertilized by spermatia of other species or their local forms. In each intergeneric or interspecific combination, experiments were repeated at least twice, in which the results were always found essentially the same. In the combination where the monoecious layers were used as female parent, the resultant data were accepted only when the self-fertilization did not occur. The followings are noticeable from the results.

| Table 2. Digital evaluation of results of crossing in the genus *Porphyra*, through successive stages of the first generation and the following development of *Conchocelis*. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Dioecious ♂      | Monoecious ♂    |                 |                 |
| P               | U               | A               | T               | Tk              | Tp              | Y               |
| P               | —               | 22222           | 23222           | 12222           | 22222           | 11              |
| U               | 23222           | —               | 23232           |                 |                 |                 |
| A               | 22232           | 22222           | —               | 22222           | 12222           |                 |
| Monoecious ♀    |                 |                 |                 |                 |                 |                 |
| Tk              | 12222           | 22222           | —               |                 |                 |                 |
| Tg              | 12222           | 22232           | 22222           |                 |                 |                 |

In each series of digits in the above table, component 1 represents a result evaluated as poor, 2 as usual, and 3 as vigorous, (O none), in a successive stage of development which is arranged from left to right in such order as:

- First: beginning of *Conchocelis* development,
- Second: further growth of *Conchocelis*,
- Third: conchospore formation,
- Fourth: growth of thallus of the next generation, where an asterisk shows abnormality,
- Fifth: formation of sexual cells and following *Conchocelis* development, where an asterisk shows absence of spermatium.

1) Unexpectedly, from almost every combination tried, carpospores developed and grew into *Conchocelis*.

2) With the *Conchocelis* normally growing, conchospores abundantly formed on them, normally liberated themselves, and began to germinate into leafy thalli.

3) In both combinations of monoecious ♀ × monoecious ♂ and dioecious ♀ × dioe-

1. P. angusta (D) ♀ × P. umbilicalis? (D) ♂
2. P. tenera? from Mangoku Inlet (M) ♂ × P. tenera (M) ♂
3. Young abnormal thalli of P. tenera (M) ♀ × P. angusta (D) ♂. Note apical parts of the two thalli in the left that look normal.
4. Abnormal thalli of P. pseudolinearis (D) ♀ × P. tenera? from Kesennuma Bay (M) ♂
5. Normal dioecious thalli of P. angusta (D) ♀ × P. tenera? from Kesennuma Bay (M) ♂
6. Normal monoecious thalli of P. pseudolinearis (D) ♀ × P. tenera? from Kesennuma Bay (M) ♂
cious, the leafy thalli normally grew and produced sexual cells, which, after fertilization, developed into Conchocelis of the next generation. The leafy thalli had intermediate characters of both parent thalli, but rather resembled that of female parent (Plate I, 1-2).

4) In the combination of monoecious × dioecious, a high mortality was found at stages as early as of two to five cells in leafy thalli germination. Only 2 to 20 per cent of the germlings survived, and the rate seemed to vary depending on combinations. Afterwards, survivals grew at a usual rate into mature thalli of three groups obviously distinguishable from one another.

Among them, the first and the second groups consisted of dioecious and monoecious thalli, respectively, and were generally somewhat different from their parent layers (Plate I, 5-6). They formed sexual cells, which, after fertilization, produced Conchocelis of the next generation. It was found, that most of the leafy thalli grown up from the Conchocelis were the very pictures of their parents, but in some crossings, abnormal thalli as described later were found appearing intermingled.

Table 3. Composition of the first descendant thalli, resultant from crossing of dioecious × monoecious.

<table>
<thead>
<tr>
<th>Combination</th>
<th>Thalli composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dioecious</td>
</tr>
<tr>
<td>Dioec.♀ × Monoec.♂</td>
<td></td>
</tr>
<tr>
<td>A♀ × Tp♂</td>
<td>-</td>
</tr>
<tr>
<td>A♀ × Tk♂</td>
<td>-</td>
</tr>
<tr>
<td>A♀ × Tg♂</td>
<td>-</td>
</tr>
<tr>
<td>A♀ × Y♂</td>
<td>-</td>
</tr>
<tr>
<td>P♀ × Tp♂</td>
<td>-</td>
</tr>
<tr>
<td>P♀ × Tk♂</td>
<td>-</td>
</tr>
<tr>
<td>Monoec.♀ × Dioec.♂</td>
<td>-</td>
</tr>
<tr>
<td>Tk♀ × A♂</td>
<td>*</td>
</tr>
<tr>
<td>Tg♀ × A♂</td>
<td>-</td>
</tr>
</tbody>
</table>

* Occurred not as an individual, but as an apical part of the abnormal thallus.

The third group usually dominated in number and was composed of obviously abnormal thalli, with a dark color. The thalli were often constricted at places, where

Fig. 1. Young abnormal thallus from the crossing of *P. pseudolinearis* (dioecious) ♀ × *P. tenera*? from Kesennuma Bay (monoecious) ♂. ×70.

Fig. 2. Vegetative cells of abnormal thallus from the crossing of *P. pseudolinearis* (dioecious) ♀ × *P. tenera*? from Kesennuma Bay (monoecious) ♂. ×250.
rhizoidal cells were seen (Plate I, 4). Microscopically the cells were a little larger and had vacuoles much larger than those in the cells of normal thalli (Fig. 2). A majority of the thalli had no spermatium forming on them, but had cells that looked like carpogonia with trichogyne-like protuberances. Those cells, without receiving spermatia, developed into groups of carpospore-like cells (Fig. 3), which, in turn, germinated into Conchocelis. The Conchocelis normally grew, formed conchospores, and repeated the same course of life.

In some combinations, normal monoecious or dioecious part was observed forming at the tip of the said abnormal thalli (Plate I, 3).

Discussion

1. On the fertilization

Mature lavers form carpogonia and spermatia, to be sure. But incredulity has often arisen as to a question if they are really fertilized at all. Drew claimed that if the so-called cystocarps should have all resulted from fertilization, the number of potential carpogonia should have been much greater than she could reasonably estimate. She also pointed out disagreement among the fertilization courses reported by previous authors. Based on cytological studies Krishnamurthy asserted that no fertilization would occur in Porphyra umbilicalis in England.

As shown in Table 1, no carpogonia of the three dioecious species independently developed into Conchocelis. It was only after their contact with spermatia that carpogonia developed to carpospores, which grew into Conchocelis. The fact proves that the fertilization did occur as far as these works were concerned. The same is most probably true with the monoecious lavers, though the self-fertilization somewhat confused the results.

In regard to the fertilization process different observations, as Drew indicated, have been reported. Among them Kunieda maintained that a spermatium is ingulfed into carpogonium at the trichogyne. Fujiyama et al. (unpublished) have confirmed Kunieda’s view through their detailed observations. The present author has very frequently observed the same process as his, too.

2. On the Crossing

In Table 2 showing the results from the intergeneric experiments, a question may remain as to if a crossing took place, in the strict sense of the term, between a pair-
of different species, i. e., whether or not the carpogonia could parthenogenetically develop when being stimulated by spermatia but without through proper fertilization.

The doubt, however, can be solved on the evidence as in Table 3 showing that the combination of dioecious × monoecious was capable of producing three different groups of thalli, i. e., dioecious, monoecious, and abnormal. It can be safely claimed, therefore, that in Porphyra intergeneric crossings can easily occur between, at least, a pair of some different species. It is unanimous among many cytological works that the leafy thalli are most probably haploid. Then, a logical deduction is that segregation, while it occurs in the diploid plants at the second filial generation, must immediately occur in the leafy thalli of the first generation. This was confirmed by the occurrence of the three different groups of thalli from the dioecious-by-monoecious crossing. The abnormal thalli, which had larger cells with larger vacuoles than usual, showed a high mortality at germination, and upon maturing, formed no spermatium, but carpogonia that developed into Conchocelis without fertilization. These phenomena are understandable if the hybrid thalli are of a “heterodiploid” nature, having haploid chromosomes from the both parents together in their cells. Of course, the assumption remains to be cytologically examined in future.

3. On the Life History

A question remains obscure as to what point of the life history of the layers is responsible for the reduction division. In this regard, one may remember that a critical mortality in the dioecious-by-monoecious crossing took place no where but shortly after the beginning of conchospore development. The fact, in the absence of evidences indicating contrarily, may suggest occurrence of the meiosis immediately before the germination of conchospores.

Many workers (see Drew6) have been of opinion that the carpospores or alike cells can develop into leafy thalli. As shown in Table 1, the unfertilized carpogonia can develop through carpospore-like cells into leafy thalli. It will be accepted, therefore, that the fertilized carpogonia develop only into Conchocelis, and that the unfertilized ones are sometimes able to originate leafy thalli.

4. On the Classification

It may be warranted to recognize that the three dioecious species studied are in close relation with one another. Because, as far as these species are concerned, crossing readily occurred, the thalli in the next generation had intermediate characters of their parents, and the hybrid was capable of normal sexual reproduction. In the first hybrids from the dioecious-by-monoecious crossing, however, high mortality and abnormal characters suggest existence of a decisive difference between the chromosomes of the parent layers.
5. Recommendations

The success of the present work seems to assure possibilities where crossing will produce new lavers more suitable for the commercial culture; furthermore, careful selection will lead to a hitherto unknown laver which, though man might have overlooked it, could probably occur, with commercially favorable characters, due to crossing in either nature or culture or both.

Under the present circumstances of technical and other factors, it is tentatively thought that the selection will be more practicable than artificial crossing in laboratory to introduce a new and commercially advantageous laver into the industry.

Summary

Artificial crossings were tried between five species including four local forms of the genus Porphyra.

The fertilization is followed by carpospore formation and Conchocelis development. Most of unfertilized carpogonia became decay, but some of them developed into carpospore-like cells which germinated leafy thalli. Therefore, the fertilization was confirmed by observing Conchocelis-development.

Crossings easily occurred between any pair of Porphyra species tested. The descendants both from dioecious parents and from monoecious parents grew normally, while those from the dioecious and monoecious combination died in mass at their young stage, though some of them survived into three different groups of thalli: dioecious, monoecious, and abnormal. The abnormal thalli are assumably of a "heterodiploid" nature, having haploid chromosomes from the both parents together in their cells.

Discussion was made in regard to process of fertilization, the moment of the meiosis in the life cycle, the relationship between Porphyra species studied, and the application of the results to the industry.

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References