

Intraspecific sterility barrier confirms that introduction of *Sphaerotrichia divaricata* (Phaeophyceae, Chordariales) into the Mediterranean was from Japan

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Abstract

Crossing studies revealed an intraspecific sterility barrier on the level of zygote formation between Japanese *Sphaerotrichia divaricata* and isolates of the same species from the Northeast Pacific and the North Atlantic. Because no consistent morphological differences exist between sporophytes from Japan and other areas, we propose not to distinguish the intersterile populations as different species. Japanese *Sphaerotrichia* and isolates from a recently detected population in the Étang de Thau, French Mediterranean coast, are interfertile. The crossing studies support the assumption that this Mediterranean population is a recent introduction from Japan.

Introduction

In the Étang de Thau (Hérault, France), a shallow basin connected by two channels with the north-western Mediterranean, a number of alien seaweeds appeared at the beginning of the last decade. Among them, there were species endemic to the north-west Pacific Ocean, such as *Undaria pinnatifida* (Harvey) Suringar (Boudouresque *et al.*, 1985) and *Laminaria japonica* Areschoug (Riouall, 1985). It was assumed that they were introduced from Japan, because oyster cultivators in the Étang de Thau had received oyster spat of *Crassostrea gigas* (Thunberg) from Japan until 1980 (Riouall, 1985). However, some of the newly recorded species, for example, *Desmarestia viridis* (O. F. Müller) Lamouroux (Verlaque,

1981), *Chorda filum* (Linnaeus) Stackhouse, and *Sphaerotrichia divaricata* (C. Ag.) Kylin (Riouall, 1985) also occur in temperate Europe, and there exists the alternative possibility that they have been transported or migrated to the Mediterranean from the Atlantic.

Sphaerotrichia divaricata occurs on cold-temperate coasts of the northern hemisphere, on both sides of the Atlantic and Pacific Oceans (Fig. 1; Kang, 1966; South & Tittley, 1986; Scagel *et al.*, 1989; Yoshida *et al.*, 1990). It has a heteromorphic life history. The sporophyte is seasonal and up to 50 cm in length. In colder regions, it appears in April and May and persists until autumn (Perestenko, 1980; Novaczek & McLachlan, 1987). In the Étang de Thau it occurs from May to July (Riouall, 1985). During the rest of the year the

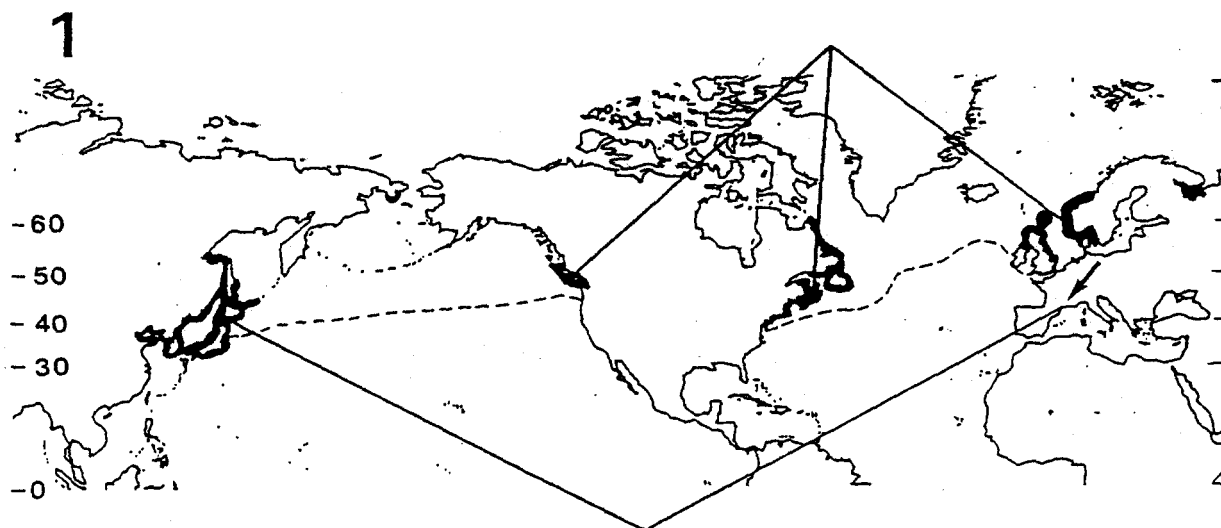


Fig. 1. World-wide distribution of *Sphaerotrichia divaricata* (bold parts of coast; arrow points to introduced population in the western Mediterranean) and pattern of interfertility (connecting straight lines) among the different populations. Dashed line: 10 °C surface sea water isotherm for April (US Navy, 1981).

species survives in the form of microscopic gametophytes (Hygen, 1934; Ajisaka & Umezaki, 1978). Gametes, zygotes, and thus new sporophytes are formed in *Sphaerotrichia* only in long days at temperatures below 15 °C (Peters *et al.*, 1987). This combined temperature and long-day photoperiodic effect confines *Sphaerotrichia* to cold temperate regions (Novaczek & McLachlan, 1987). Except for the new finding in the Étang de Thau, *Sphaerotrichia* is not recorded in Europe from south of the Channel Islands (Hamel, 1931–39, as *Nemacystus divaricatus* (C. Ag.) Kuckuck). A record from western Turkey (Güven & Öztiğ, 1971) was apparently a misidentification. *S. divaricata* is not included in a more recent flora of that region (Güner *et al.*, 1985).

Six species have been described in the genus *Sphaerotrichia*. Zinova (1958) recognized three of them. Using overall morphology and cortical anatomy she distinguished *S. disessa* (Setchell et Gardner) Zinova (based on *Chordaria disessa* from Washington, eastern Pacific) and *S. firma* (Gepp) Zinova (based on *Chordaria firma* from northern China) from the type species of the genus. However, Inagaki (1958) and Perestenko (1980) proposed to merge all taxa of *Sphaerotrichia* in one species. Peters *et al.* (1987) demon-

strated zygote formation among gametophytes from Europe and Atlantic and Pacific North America. This was taken as evidence supporting the merger proposed by Inagaki and Perestenko.

Crossing studies using additional isolates from Japan and the Étang de Thau are described in the present paper. They revealed an unusual pattern of intersterility among the world-wide populations of *Sphaerotrichia* and provided experimental evidence that the population in southern France was introduced from Japan.

Material and methods

Previously studied male and female gametophyte clones of *Sphaerotrichia divaricata* (described in Peters *et al.*, 1987) were from Bamfield, British Columbia and Pomquet Harbour, Nova Scotia. From northern Europe we had a single female gametophyte from Isefjord, Denmark. New collections (Table 1) were made in Hokkaido (northern Japan) and at Bouzigues, Étang de Thau, southern France. Sporophytes with unilocular sporangia were utilized to start cultures. Clonal cultures were obtained by separating single germ-lings from mixed gametophyte cultures. Clonal

Table 1. Collections of sporophytes of *Sphaerotrichia divaricata* in Japan and the western Mediterranean, and gametophyte clones isolated. Except for the first collection from Hokkaido, only a single gametophyte was isolated from each collected field sporophyte from Japan. f = female; m = male.

Locality	Date (d-m-y)	Sex of clones
Oshoro, West Hokkaido	26-08-87	5 × f
Oshoro	12-07-88	1 × m
Oshoro	14-07-88	1 × f
Oshoro	29-07-88	1 × f
Shiraiwa, Hokkaido	11-08-88	1 × m
Notsukamappu, E. Hokkaido	28-08-88	1 × m
Bouzigues, Étang de Thau	16-06-90	2 × f, 2 × m
Bouzigues	16-06-90	1 × f, 1 × m

gametophyte cultures were subjected to a set of culture conditions varying in regard to temperature and daylength (Table 2), whereas light was stable at $30 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, from daylight-type fluorescent tubes. Sex of the gametophyte clones was determined according to the mating behaviour of the respective gametes by observing plasmogamy.

For the crossing studies one pair of gametophytes was selected from each geographical region and the female gametophyte from Denmark. They were cultivated for two weeks in long days at 10°C . Addition of fresh culture medium then stimulated formation of gametes which were released after 5–6 days. Crossing studies were made by mixing zoids of two different strains, and two strains were regarded as compatible when they showed gamete fusions.

Table 2. Culture conditions and developmental responses of gametophytes of *Sphaerotrichia divaricata* from Japan. g = gametes, s = asexual swimmers produced. SD = 8:16 h light:dark; LD = 16:8 h light:dark. ND = no data.

	SD	LD
5°C	s	g
10°C	s	g
15°C	s	g
20°C	s	ND

Assimilatory filament length, number of cells in assimilatory filaments, and diameter of the globular end cell of assimilatory filaments were measured in 56 fresh or rehydrated dried thalli from northern Europe, Atlantic and Pacific North America, Japan, Siberia, and China. The holotypes of *Chordaria firma* and *Chordaria disessa* were studied in the herbaria of the British Museum (Natural History; BM) and of the University of California at Berkeley (UC), respectively.

Results

Cultures. Gametophyte clones from Hokkaido were unisexual. Because from five of six field sporophytes only a single gametophyte clone was isolated, it was impossible to determine whether the field plants were heterozygous or not (Table 1). Male and female gametophytes were morphologically indistinguishable. They consisted of prostrate branched filaments 4–5 μm in diameter. Cells contained 2–5 discoid plastids with pyrenoids. Fertile female gametophytes emanated a strong pheromone odour that was lacking in vegetative or male cultures. Gametes were $3\text{--}4 \times 4.5\text{--}6 \mu\text{m}$, and sexual reproduction was isogamous. Zygotes were 7–8 μm in diameter and contained at least two stigmata.

Clonal gametophytes were also isolated from each of two sporophytes from the Étang de Thau. Both plants were heterozygous giving offspring consisting of male and female gametophytes (Table 1). They were similar to the Japanese gametophytes in vegetative and reproductive morphology, and in gamete size.

Ecophysiology. Gametogenesis in the Japanese strains occurred only in long days at temperatures from 5 to 15°C . However, the combination of long days and 20°C was not tested (Table 2). Mature gametes were released at the onset of daylight. Distribution of settled zoids in the culture dishes and movement of zoids under lateral illumination consistently showed that they were negatively phototactic.

Crossing studies (Table 3). Neither male nor female gametes from Japan were able to fuse with sexually opposite gametes from British Columbia,

Table 3. Cross-fertility studies among gametes of *Sphaerotrichia divaricata* from Japan (J), British Columbia (BC), Nova Scotia (NS), Denmark (DK), and the Étang de Thau, western Mediterranean (ET). + : plasmogamy; - ; no plasmogamy.

	♀	J	BC	NS	DK	ET
♂						
J		+	-	-	-	+
BC		-	+	+	+	-
NS		-	+	+	+	-
ET		+	-	-	-	+

Nova Scotia, or Denmark. However, they were interfertile with the gametes from the Étang de Thau. Gametes from the Étang de Thau were intersterile with gametes from British Columbia, Nova Scotia, and Denmark. Interfertility on the level of plasmogamy among gametes from British Columbia, Nova Scotia, and Denmark (Peters *et al.*, 1987) was confirmed (Fig. 1).

Morphology of field sporophytes. Thalli were up to 50 cm in length, terete, irregularly branched, and axes measured 0.5–1.6 mm in diameter. Older plants were coarser than younger individuals. Average assimilator length (including end cell) was 62 (NW America) to 88 (N Europe) μm , average number of cells per assimilatory filament was 3.6–4.75, and mean diameter of assimilatory end cells was 19.5 (NW America) to 27 (Europe) μm . Data from fresh field material and dried herbarium specimens from the same region were not significantly different. There were some significant differences among the four regions in end cell diameter, assimilator length, and number of cells per assimilatory filament. However, the eastern

Asian thalli were in all cases similar to the European thalli (Table 4).

At North Rustico, Prince Edward Island, Eastern Canada, 25 thalli were collected in a population of *Sphaerotrichia divaricata* from the beginning to the end of the season. In each thallus, overall length and 25 assimilatory filaments were measured. There was evidence of change in the proportional distribution of assimilatory cell number as plants developed. In plants of all sizes the majority of filaments was 3-celled (including end cell). However, plants less than 5 cm tall had relatively more 2-celled assimilators whereas plants over 10 cm had a greater proportion of 4-celled assimilators. There was no change associated with onset of fertility, *i.e.* between thalli <20 and >20 cm in length. Assimilator end cells were rather homogeneous, being as large in immature plants as in mature ones.

Discussion

Our present observations on the gametophytes of Japanese *Sphaerotrichia* agree in morphological details with previous results by Ajisaka & Umezaki (1978). However, we cannot confirm their observations that sexual reproduction is 'either isogamous or anisogamous', that 'gametophytes are sometimes monoecious', and that 'two swimming gametes accidentally fused on their heads'. In our cultures, there were no consistent differences in size between gametes of the different sexes, our clones were consistently either male or female, and gamete fusions occurred, as in other Phaeophyceae, after a female gamete had settled. We were not able to detect a particular

Table 4. Assimilator length, cell number, and diameter of endcell of assimilatory filaments in natural populations of *Sphaerotrichia divaricata*. EUR = thalli from northern Europa; EAST = from NE America; WEST = from NW America; ASIA = from Japan, Siberia and China. Unit = μm . Values are given as minimum (mean \pm standard deviation) maximum.

	EUR ($n = 12$)	EAST ($n = 25$)	WEST ($n = 4$)	ASIA ($n = 15$)
Endcell diameter	18(27 \pm 3.6)33	20(24.6 \pm 2.3)33	15(19.5 \pm 4.2)25	18(25.2 \pm 5.1)35
Assimilator length	50(88.9 \pm 17)130	50(66.7 \pm 17.7)125	50(62 \pm 10.3)75	50(85.1 \pm 23.5)150
Number of cells per assimilator	3(4.5 \pm 0.8)6	3(3.6 \pm 1.0)7	4(4.75 \pm 1)6	3(4.5 \pm 1.2)8

attachment site for the male gamete in the formerly anterior part ('head') of the female gamete.

Most characters of gametophytes and gametes of Japanese *Sphaerotrichia* compare well with the same characters in isolates from other areas (Peters *et al.*, 1987). Gametogenesis in Japanese *Sphaerotrichia* similarly occurs only in long days. However, temperature requirements for gametogenesis may not be the same as in the previously studied isolates. The southern distribution boundaries of *Sphaerotrichia* on Atlantic and eastern Pacific coasts coincide with the 10 °C isotherm for April rather than with the 15 °C isotherm which would be expected according to laboratory culture studies (US Navy, 1981; Fig. 1; Peters *et al.*, 1987). In eastern Asia, the southern limit lies further south indicating a higher temperature boundary for gametogenesis. Gametes in Japanese *Sphaerotrichia* have the same size as in isolates from America and Europe, but the latter are positively phototactic (confirmed in the present study), whereas Japanese and Mediterranean gametes are negatively phototactic at the same photon fluence rate (20–30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$).

There is an intraspecific sterility barrier between Japanese strains and those from Pacific and Atlantic America and northern Europe. This is particularly interesting in the Pacific where oceanographic conditions would theoretically allow *Sphaerotrichia* to occur continuously from northern China to Washington (US Navy, 1981). However, *Sphaerotrichia* is not recorded from Alaska (S. Lindström, pers. commun.). The incompatibility between the Japanese and American Pacific isolates indicates a geographical separation and different evolution of the two populations. No sterility barrier regarding zygote formation exists among isolates from both sides of the North Atlantic Ocean and from NW America. In other seaweed species tested for interoceanic interfertility, there is either no sterility barrier on the level of plasmogamy between Atlantic and NW Pacific isolates (Bolton *et al.*, 1983; Müller & Kawai, 1991) or a sterility barrier exists between NE Pacific and Atlantic isolates (van der Meer & Bird, 1985; tom Dieck, 1992). Sterility barriers of sexually compatible strains may become evident

during the development of hybrid sporophytes or during meiosis (tom Dieck, 1992; Stache, 1991). However, such more elaborate sterility barriers were not evaluated in this study.

The distinction of three species of *Sphaerotrichia* recognized by Zinova (1958) was based on differences in habit, which is of little use in the highly variable Chordariales, and on differences in size and morphology of assimilatory filaments (Table 5). The type specimen of *S. disessa* was not examined by Zinova. Our measurements on several plants from Europe, Nova Scotia, Washington, Japan, Siberia and China (Table 4) show that the geographically separated populations of *Sphaerotrichia* widely overlap in the characters assimilator length, cell number and dimensions of the assimilator end cell. Our measurements confirmed that the type specimen of *S. firma* from Qingdao (in BM) has unusually small assimilator end cells and an unusually large number of cells per assimilatory filament (Table 5). However, specimens in UC from China including Qingdao (UC # 555127 and 776145), from Japan ($n = 7$) and Siberia ($n = 7$) agree with specimens from the Atlantic. The distinction of the different taxa proposed by Zinova does not seem to be justified.

Although sexual incompatibility and the differences observed in culture (negative vs. positive phototaxis of gametes) indicate genotypic separation within *Sphaerotrichia*, only non-cryptic and consistent – thus practical – morphological differences would require and justify taxonomic distinction. For the moment we propose not to consider the studied northern Japanese *Sphaerotrichia* as taxonomically different from *S. divaricata*. We intend to examine more isolates from central and southern Japan, including the type locality of

Table 5. Assimilator length, cell number, and diameter of endcell of assimilatory filaments in taxa of *Sphaerotrichia* recognized by Zinova. From Zinova (1958). Unit = μm .

	<i>S. divaricata</i>	<i>S. disessa</i>	<i>S. firma</i>
Endcell diameter	23	23–45	13–20
Assimilator length	80–120	50–83	82–210
Number of cells per assimilator	5–8	2–4	5–9

S. sadoensis Noda more recently described from Sado Island, Japan (Noda, 1975), and isolates from Korea, China and Siberia to examine whether all populations of *Sphaerotrichia* from the Northwest Pacific are similar and interfertile or not.

Our crossing studies strongly support Riouall's (1985) assumption that *S. divaricata* was introduced into the Étang de Thau from Japan. The species seems to have established well at Bouzigues where we found it not only at the entrance to the harbour (locality given by Riouall) but also in large numbers on the small beaches west of the village, where it occurred in 0–1 m depth. Because male and female gametophytes were isolated from our material, the original introduction must have included a heterozygous sporophyte or gametophytes of both sexes.

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