Supplementary information

Addition of sporulation inhibitor 1 (SI-1) prevents gametogenesis of *U. mutabilis* in culture

Materials and Methods

Purification of the sporulation inhibitor (SI-1) and bioassay

Extraction. Stratmann et al. (1996) established extraction protocols for the SI-1, which were slightly modified and applied *U. mutabilis* (Kessler et al., 2018; Vesty et al., 2015). 500 mL of the *Ulva* culture medium (UCM) from 2–3 week old axenic *U. mutabilis* cultures was stirred with 50 mL phenol (saturated with 100 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} EDTA, pH 7.5) in a 1 L two-neck round-bottom flask for 20 minutes at 20 °C to extract the SI-1 from the UCM. The phenol phase was transferred into plastic tubes after centrifugation (3800 g, 10 minutes). The extraction process was repeated once more, and the phenol phases were combined. After re-extracting with 100 mL of 10 mmol L-1 Tris-HCl (pH 8.0), the phenol phase was mixed with three volumes of acetone and incubated at -20 °C for 30 minutes. Centrifugation (3800 g, 20 min, 0 °C) was used to collect the precipitate, washed three times with pre-cooled ethanol (-20 °C). After lyophilisation, the precipitate was suspended in 100 mmol L^{-1} Tris-HCl (pH 8.0) and stored at -20 °C. A dilution series tested the activity of the partly purified sporulation inhibitor.

Estimation of SI activity. *Ulva* fragments were washed twice in a fine sieve after being chopped. The fragments ($N_{Fragments\ (total)} = 100 \pm 30$) were transferred into 96 multiwell dishes (Nunc, Roskilde, Denmark) containing 100 µl UCM for gametogenesis analysis. The concentration of the SI-1 was determined through a dilution series of the partially purified compounds with UCM. (Due to the nature of the discrete dilution series, measurement variance is also affected by the interval between dilution steps).

$$Inhibitory\ rate\ (\%) = \frac{N_{Fragments\ (total)} - N_{Fragments\ with\ gametangia}}{N_{Fragments\ (total)}} \times 100$$

One unit of the SI is defined as the minimal amount of the factor that inhibits differentiation of a blade cell into gametangia with an *Inhibitory rate* of 50% in 1 mL UCM within three days under standard conditions(Stratmann et al., 1996). 10 Units were applied in the bioassay.

Bioassay. The gametogenesis of a mature thallus can be induced by changing the UCM and opening the tube(Alsufyani et al., 2017). Those thalli were used to test the activity of the sporulation inhibitor, ten

units of the extracted SI-1 from the *Ulva* culture medium of a young *Ulva* population (100/0) were added to one Petri dish, while the other part of the thallus was simply incubated in UCM.

Results

Purified SI-1 was added to mature and induced *Ulva* thalli (**Fig. S1**), demonstrating that SI-1 can be taken up by *Ulva* and used to regulate gametogenesis of cultures of the 100/0 group. We thus conclude that the sporulation event is delayed or even inhibited in age-mixed cultures composed of young, smaller thalli and old, larger thalli. The bioassay backs up the conclusion of the model that age-mixed populations are more stable than uniform ones.

Figure S1. Inhibition of gametogenesis by an externally supplied sporulation inhibitor (SI-1). Mature gametophytes will undergo gametogenesis if the SI is removed (thallus #1,2) were transferred into fresh UCM distributed in two Petri dishes. Within three days gametes were formed and released (left). The addition of 10 units of SI-1 inhibited the differentiation of thallus cells into gametangia (right).