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Short Communication

Cell Fusion in *Dinophysis fortii* Pavillard

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Abstract The process of cell fusion in *Dinophysis fortii* was examined on the basis of time course culture observations. Ten days after inoculation, dwarfish cells began to appear. Dwarfish cells paired with normal cells of the same shape and size as vegetative cells. The two cell types were connected by a thread-like object. The dwarfish cell then gradually invaded the normal cell near a flagellar pore. Cell fusion was completed within 20 min after the beginning of invasion. The shape of the cell which completed cell fusion apparently did not differ from a vegetative cell shape. We discuss the cell fusion process in relation to the possibility of sexual conjugation.

Key words: *Dinophysis fortii*, cell fusion, dwarfish cell, sexual reproduction, cannibalism

The genus *Dinophysis* is known as a dinoflagellate group that causes diarrhetic shellfish poisoning (DSP). The physiological and ecological characteristics of this taxa are not yet fully understood due to difficulties in culturing the organisms. Sexual reproduction has been poorly documented in the life cycle of *Dinophysis* spp. MacKenzie (1992) reported the possibility of sexual reproduction in *Dinophysis* spp., based on observations of wild populations. McLachlan (1993) observed the presumed sexual stage of *D.* cf. *acuminata* in which cells have two trailing flagella. *Dinophysis fortii* Pavillard, which is a species responsible for DSP in the Japanese coastal waters (Yasumoto *et al.* 1980), has been shown by Fukuyo (1993) to have a presumed sexual stage during which cell fusion was observed between normal sized and dwarfish cells. We observed a process of cell fusion in this DSP causing dinoflagellate through several laboratory culture trials. This paper describes a cell fusion process in *D. fortii*, which appeared to be a sexual process based on the time course observations.

D. fortii cells were isolated monoalgally from seawater collected at Gokasho Bay, Japan on June 6, 1995. Cultures were established in wells of a 24 well plate (Sumitomo Bakelite Co., Ltd) containing 2 ml of f/2 medium (Guillard and Ryther 1962). Three individuals were inoculated into the medium at the start of culture. Small dinoflagellates, *Heterocapsa triquetra* and *Gymnodinium* sp., were added to each well at cell densities of 100-500 cells/ml as a food source, assuming that this species is mixotrophic since some species of the genus *Dinophysis* are known to be heterotrophic (Hansen 1991, Jacobson and Anderson 1994). The strains of *H. triquetra* and *Gymnodinium* sp. were the same as used previously (Uchida *et al.* 1997). Culture experiments were conducted at 20±1°C under a photoperiod of 12 h L : 12 h D with illumination from cool – white fluorescent lamps (80–120 μmol/m^2/s). Morphological observations were carried out under an inverted microscope. When necessary, fusing cells were isolated into fresh medium for detailed observations.

D. fortii continued to grow by binary fission for over a month after the inoculation. However, growth was slow, and the maximum yield was 40 cells/well. Ten days after inoculation, dwarfish cells of 45–48 μm in length began to appear (Fig. 1a). These cells were small and slender in shape compared to the vegetative cells (62–68 μm in length). Some dwarfish cells were observed to pair with normal cells (NM cells) which did not appear to differ from vegetative cells in size and shape (Fig. 1b). A thread like object appeared to connect the two types of cells at their dorsal edges. The dwarfish cell then gradually invaded the NM cell near the flagellar pore (Fig. 1c, d). The fusion between dwarfish and NM cells was completed within 20 min. of the beginning of invasion. Post-fusion cell shape (Fig. 1e) was not markedly different from a vegetative cell shape. The fate of fused cells was not verified because we were unable to maintain them in culture. However, cells which completed the fusion were observed to maintain their motility for at least two days. The morphological features of dwarfish and paired *D. fortii* cells were almost the same as those described by

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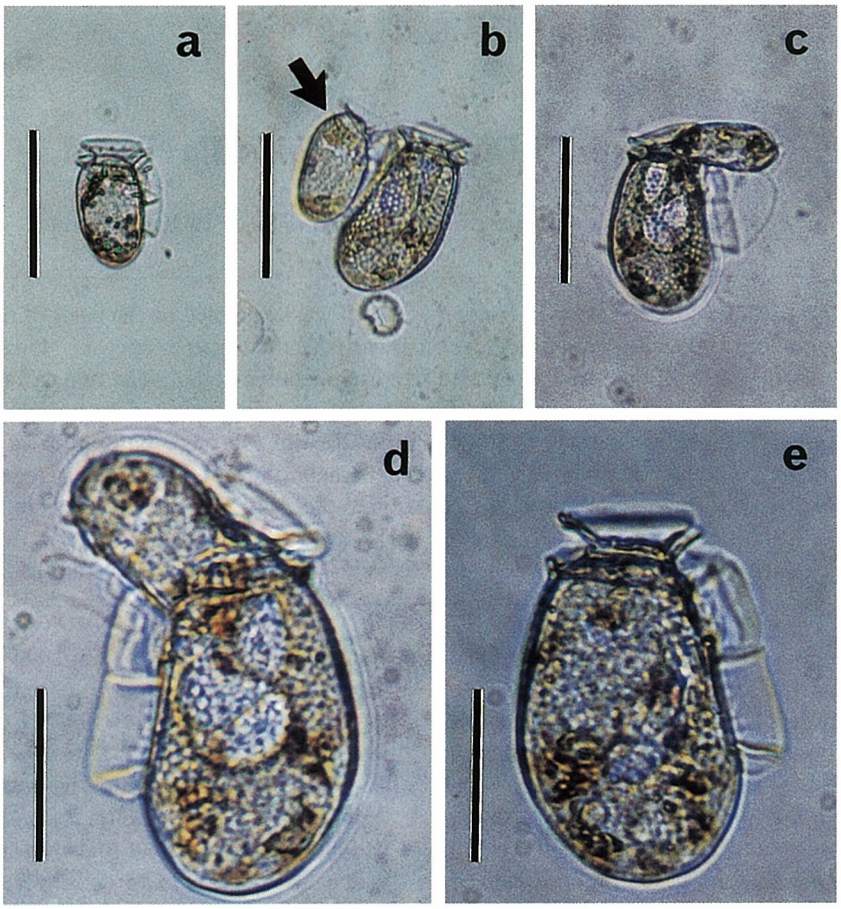

Figs. 1a–e. Process of cell fusion in *Dinophysis fortii*. **a**. Dwarfish cell. **b**. Pairing between a dwarfish cell (arrow) and a normal sized cell (NM cell). **c**, **d**. Fusion between dwarfish and NM cells. **e**. Cell after the completion of cell fusion. Bars in **a–c** show 50 μm, and those in **d** and **e** show 30 μm.

Fukuyo (1993). Furthermore, the present study succeeded in showing the process of cell fusion between dwarfish and NM cells in *D. fortii* through time course observations. MacKenzie (1992) observed couplet formation between *Dinophysis acuta* and the smaller *D. dens*, and between large and small cells of *D.* cf. *acuminata* using naturally collected seawater samples, and hypothesized that couplet formation is a process of sexual conjugation. In the former case it was likely that the smaller *Dinophysis* cells identified as *D. dens* were dwarifish cells of *D. acuta*. Cell fusion in *D. fortii* can be interpreted in two ways: sexual reproduction or cannibalism. Thecate dinoflagellates *Protoperidinium* cf. *divergens* and *P. crassipes* have been reported to feed on conspecific cells when populations are high (Jeong and Latz 1994). The cell fusion in *D. fortii* observed here may be regarded as cannibalism. If *D. fortii* is phagotrophic, cannibalism seems reasonable for the persistence of the population. However, food vacuoles originating from prey organisms were not found in the fused cells of *D. fortii* (Fig. 5). As for other species in the genus *Dinophysis*, *D. rotundata* and *D. hastata* are known to predate on ciliates by sucking prey cytoplasm through a feeding tube (Hansen 1991, Inoue *et al.* 1993). However, this behavior is far different from the cell fusion mechanism of *D. fortii* observed by Fukuyo (1993) and in the present study. Moreover, it is unreasonable from the view of growth strategy that *D. fortii* should undergo cannibalism in cultures where *H. triquetra* and *Gymnodinium* sp. cells coexist. We never observed predation by *D. fortii* on these two phototrophic dinoflagellates. Therefore, it is highly possible that cell fusion between dwarfish and normal *D. fortii* cells is a process of sexual conjugation. The dwarfish cells produced during the culture of vegetative cells may have differentiated into gametes of this species. In this case this species is regarded as anisogamous. However, further cytological studies are necessary to certify whether the fusion observed in *D. fortii* is a sexual process or not.

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培養中に観察された *Dinophysis fortii* の細胞間融合について

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下痢性貝毒の原因種である *Dinophysis fortii* を三重県五カ所湾から分離・培養したところ，栄養細胞接種後10日目から矮小性の細胞が観察され始めた。矮小性の細胞は形態的に栄養細胞と同一の細胞と結合し，やがて飲み込まれるようにこの細胞と合体した。合体を完了した個体は栄養細胞とほぼ同じ形態を保ち，単離・培養後も約２日間は活発に遊泳を続けた。このように本報告では *D. fortii* の細胞間融合についてその過程を連続観察に基づいて述べるとともに，有性生殖との関連について考察した。