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Isolation and Characterization of Feeding-Deficient Strains in Inbred Lines of the Hydrozoan Jellyfish *Cladonema pacificum*

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Feeding behavior in cnidarians has been studied as a model experimental system in physiology and neurobiology. Although the feeding response in cnidarians, such as *Hydra*, is triggered by chemical signals, the underlying molecular mechanisms that ensure their precise execution are not well understood. It could be largely due to the lack of genetic analysis in cnidarian experimental systems. *Cladonema pacificum* is a hydrozoan jellyfish that is easy to maintain and cross for genetic analysis in the laboratory. To establish *C. pacificum* as a model experimental animal in cnidarians, we have been inbreeding strains of jellyfish. Here, we document our progress in developing *C. pacificum* inbred lines and feeding-defective strains that we isolated in the course of inbreeding. In the inbred lines, an increasing number of feeding-defective strains appeared as descending generations and finally all the F5 progeny showed a feeding-deficient phenotype presumably owing to inbreeding depression. Feeding behaviors of these strains were analyzed by video microscopy and we found that the feeding-defective strains captured prey, but could not kill them. After trapping prey, wild-type medusae contracted their tentacles tightly and then bent the tentacles to bring the prey to the mouth; however, feeding-defective medusae rarely contracted their tentacles and did not bend. These feeding-defective phenotypes are caused by lack of stinging nematocytes in their tentacle batteries. These findings furnish a clue to the regulatory aspects of feeding behavior, but also reveal the mechanisms of stinging nematocyte transport in tentacles.

Key words: feeding behavior, inbreeding, nematogenesis, jellyfish, *Cladonema pacificum*

INTRODUCTION

All animals must consume food to survive and reproduce. Proper behavioral coordination and control of feeding are therefore essential. *Hydra*, jellyfish, and sea anemones belong to the phylum Cnidaria, and have relatively simple nervous systems that have evolved in parallel to those of Bilateria from a distant common ancestor. Feeding behavior in cnidarians is controlled by chemical signals, and has been studied as a model experimental system in physiology and neurobiology. The feeding response in *Hydra* is activated by reduced glutathione (GSH) and has been extensively studied (Loomis, 1955). The feeding behavior in *Hydra* is evoked by GSH released when living prey is injured by the stinging nematocytes. In *Hydra* GSH induces feeding behaviors as a consecutive sequence of the following three actions, (1) tentacle sweeping back and forth in a concerted flexion, (2) tentacles contracting towards the mouth, shorten, and writhe, (3) all tentacles bending tightly around the mouth (Lenhoff, 1961; Hanai, 1981; Koizumi et al., 1983).

Cnidarians form nematocysts, an intracellular organelle unique to animals of this phylum. Nematocysts are used for various behaviors in cnidarians, including prey capture, predator defense, and locomotive movement. Although more than 25 types of nematocyst have been identified among the cnidarians, two types of nematocyst—desmoneme and stenotele—are involved in feeding (Kass-Simon and Scappaticci, 2002). To capture prey, such as the brine shrimp *Artemia* nauplii, the desmoneme, which contains no poison, acts like a lasso in wrapping around the bristles of the prey. The stenoteles, the stinging nematocytes, pierce the prey and inject poison into the prey to kill or paralyze it. Nematogenesis in *Hydra* has been studied as a model of the neurogenetic process. In *Hydra*, nematocyte production is scattered throughout the body column ectoderm (David and Challoner, 1974; David and Gierer, 1974). The medusae of the hydrozoan species, such as *Clytia* and *Podocoryne* have a distinct nervous and muscular system compared to the polyp stages, reflecting different lifestyles and behaviors (Brusca and Brusca, 2003). In *Clytia hemisphaerica*, the ordered progression of nematogenesis from stem cells through differentiation stages in tentacle bulb, a bulge at tentacle bottom has been described in details (Denker et al., 2008).

Although feeding responses that are triggered by chemical signals in cnidarians have been studied for a long time, the underlying molecular mechanisms that control the

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responses are not well understood. We thought that this could be due largely to the lack of genetic analysis in the cnidarian experimental systems. To overcome this issue, here, we have developed an experimental system of cnidarian genetics and isolated feeding-defective strains of *C. pacificum* in the course of inbreeding. These strains were observed by video and histochemical microscopy, and we found that the feeding-defective phenotype is caused by the lack of stenotele in the tentacle batteries.

MATERIALS AND METHODS

Animals

Deguchi and colleagues established several strains of *C. pacificum* around the Tohoku area in Japan and have maintained them for more than 10 years (Deguchi et al., 2005). The entire life cycle of *C. pacificum* (Fujiki et al., 2019) can be regulated in laboratories, which enables us to obtain mature jellyfish of this species throughout the year (Deguchi et al., 2005). The mature eggs and sperms were prepared and fertilized as described previously (Deguchi et al., 2005) except using artificial seawater, Sea Life (Marine Tech Inc., Japan) instead of natural seawater.

Inbreeding in the laboratory

Jellyfish breeding in the laboratory was carried out in 6 cm dishes filled with 15 ml of artificial seawater. Typically, 10 females and 10 males were selected from the offspring obtained from a single pair of parents. To determine which pair's offspring would be intercrossed to generate the next generation, the following criteria were used: (1) the average number of eggs laid, (2) fertilization efficiency, (3) the average number of embryos surviving until primary polyps, and (4) the normal growth into healthy adult medusae.

Successive inbreeding was carried out since 2014. The founder individuals, 6W (female) and N3 (male) were obtained at Orinohama (Ishinomaki City, Miyagi Prefecture, Japan) and established by Deguchi et al. Spawning was induced by controlling light and dark conditions as described previously (Deguchi et al., 2005). The planula larvae settled to the bottom of the dish 2–3 days post fertilization (dpf). After metamorphosis primary polyps appeared 6–7 dpf. Sliced brine shrimp was fed to the primary polyps once every other day for two weeks. The primary polyps were thereafter able to catch and eat the swimming brine shrimp nauplii. Each primary polyp developed stolons and formed a colony with two or three polyps three

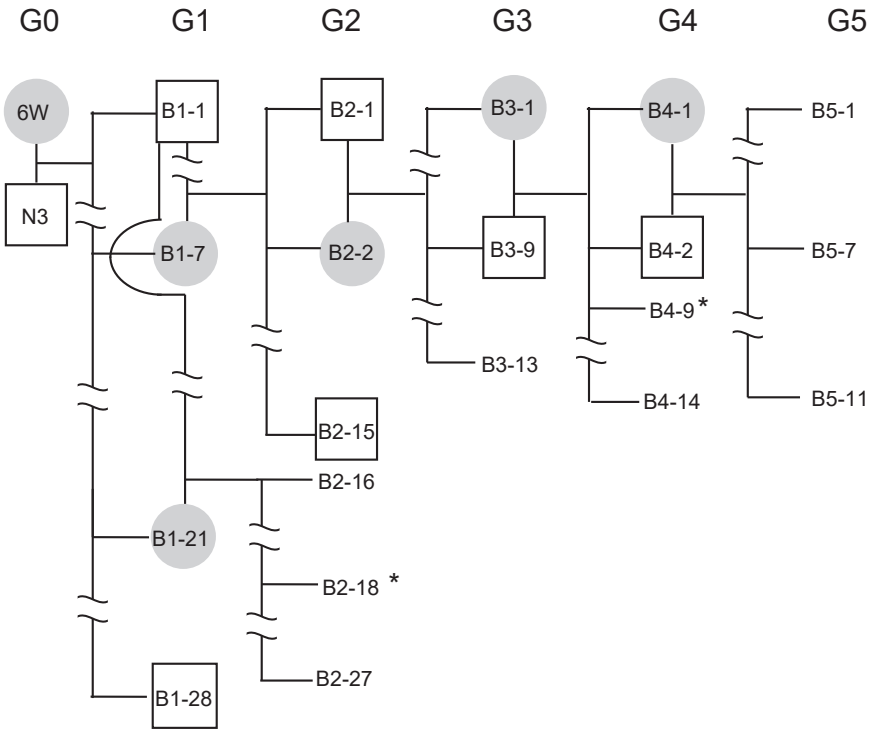


Fig. 1. Pedigrees of the jellyfish inbred lines. Asterisks show feeding-defective strains used in this study. Bolded paired identifiers represent the pair whose offspring were used for inbreeding. Shaded circles and squares show female and male, respectively. Strains whose sex could not be identified not labeled.

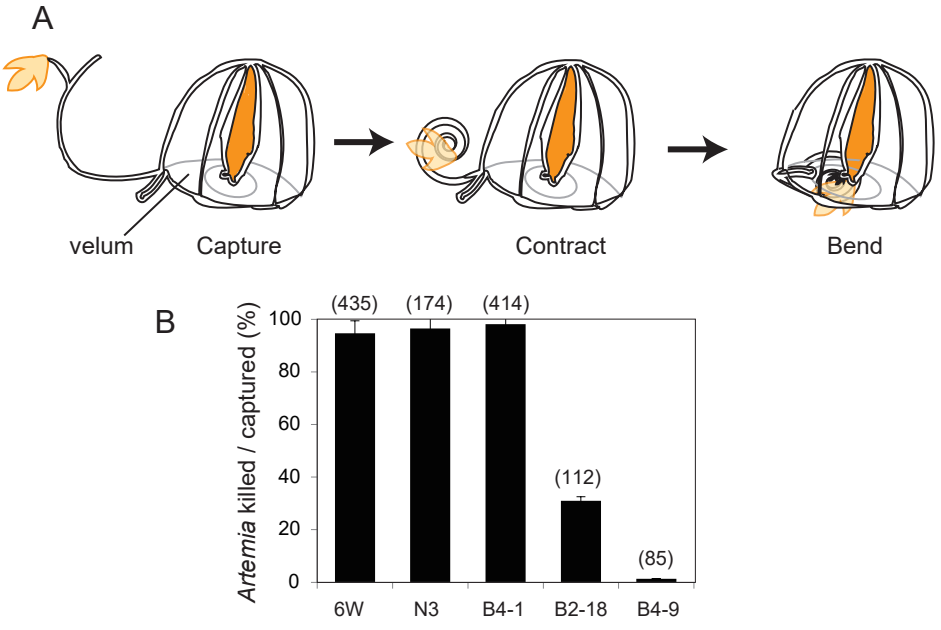


Fig. 2. Feeding behaviors of the medusae. **(A)** Components of the feeding behavior of *C. pacificum*. A tentacle captures a brine shrimp **(A, left)**. The tentacle contracts **(A, middle)**. The tentacle is bent at the base (see Fig. 6C) to put the prey into the bell across the velum **(A, right)**. **(B)** The percent of brine shrimps that were killed/captured by wild type medusae (6W, N3, and B4-1) and feeding-defective strains (B2-18 and B4-9). The total numbers of observed brine shrimps are indicated in parentheses.

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weeks after fertilization. To isolate a single clone, one single polyp from a colony was transferred to a fresh dish. The polyps of the newly formed colony cannot produce medusa unless they are treated at a low temperature (4°C) for at least three months (Fujiki et al., 2019). After treating three months at 4°C colonies were incubated at 20°C. The polyps of the colonies that treated at a low-temperature to form medusae about two weeks after the temperature shift and the medusae sexually matured within three weeks. Under these conditions, a whole life cycle of *C. pacificum* takes about 4–5 months.

Monitoring of the feeding behavior

For monitoring of the feeding behaviors, medusae after 1–4 weeks liberation were used. Each medusa was incubated in a well of a 4-well plate (Thermo Fisher Scientific, USA) was observed and recorded by SMZ-1500 stereomicroscope (Nikon, Japan) equipped DMC-G5X digital video camera (Panasonic, Japan). *Artemia* nauplii (A & A Marine Brine Shrimp Eggs, Vietnam) within 24 hours after hutching were given to a medusa in a well and movies were recorded for 1–10 min (Fig. 2B) and about 3 min (Fig. 3). The numbers of *Artemia* which were captured were counted, and *Artemia* which had stopped swimming were counted as dead (Fig. 2B). The jellyfish used were wild-type (6W, N3, and B4-1) and feeding-defective strains (B2-18 and B4-9) (Fig. 1).

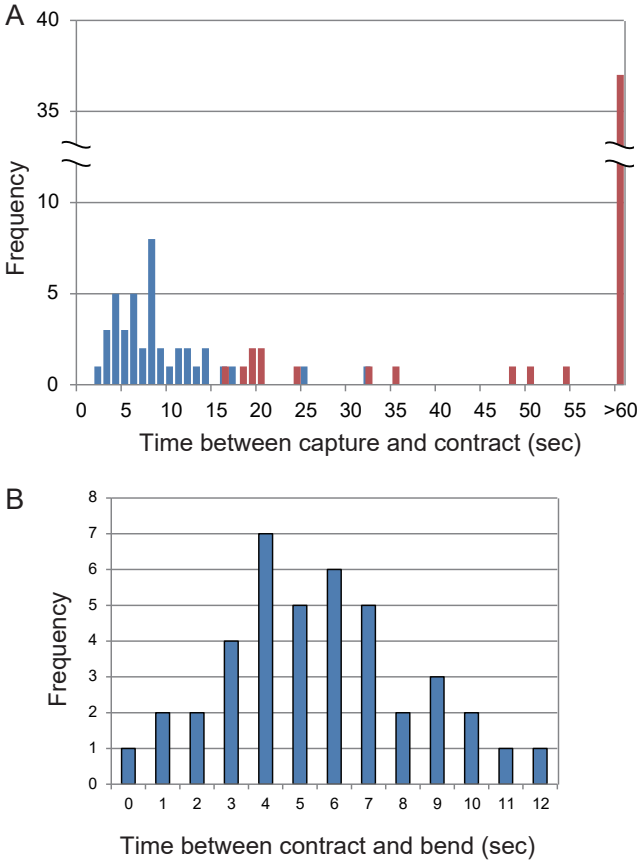


Fig. 3. Tentacle contraction and bending in wild-type and feeding-defective medusae. **(A)** Time for tentacle contraction in wild-type (6W, blue) and in the feeding-defective strain (B2-18, red). **(B)** Time for tentacle bending in wild-type (6W, blue). About 20 *Artemia* nauplii were given to a medusa in a well and movies were recorded for about 3 min. The time for tentacle contraction (see Fig. 2A, from capture to contract) for each tentacle was measured and its frequency was counted. Most of the feeding-defective tentacles did not contract or bend during the video observation (see text).

Histochemistry and microscopy

The medusae were fixed 2 h at room temperature in 4% paraformaldehyde (Nacalai tesque, Japan). The medusae were then dehydrated in ethanol and acetone and embedded in Technovit 8100 resin (Heraues-Kulzer, Wehrheim, Germany). The sections of medusa were cut at a thickness of 10 μm, stained with hematoxylin-eosin, washed in tap water, treated with xylene, and mounted with MOUNT QUICK mounting media (Daido Sangyo, Japan). The specimens were observed and photographed with Akiophot 2 (Zeiss, Germany) equipped a CCD camera CC-600L (Pixcela, Japan).

RESULTS

Inbreeding

We attempted to inbreed a wild-type *C. pacificum* population at Orinohama through full-sib mating (see Materials and Methods). The inbreeding of the Orinohama strain, 6W (female) and N3 (male), was initiated with 10 independent single-pair mating. The first obstacle to inbreeding arose in generation 2 (F2). All progeny from the cross between B1-21 (female) and B1-1 (male) exhibited feeding-deficient phenotype (Fig. 1, Table 1). Most of these feeding-deficient polyps died within two weeks after fertilization and could not form colonies. Colonies of *C. pacificum* developed from the primary polyps require to be incubated at 4°C for at least three months to form medusae (Fujiki et al., 2019). Incubation at 4°C to induce medusae is called 'low-temperature treatment'. After low-temperature treatment, eight of the 12 colonies from the cross died. Most of the survivors produced few medusae or could not produce medusae at all. Only one B2-18 strain could produce an appropriate number of medusae, however, the medusae from B2-18 showed feeding-deficient phenotype as described later (Supplementary Movie S2). All progeny including B2-18 from the cross between B1-21 and B1-1 could not form any medusae that became mature to produce gametes. Thus, we could not proceed to inbreed using the line from B1-21 and B1-1. In

Table 1. *Generation-based feeding deficiency in inbred lines of C. pacificum.* In generation 1 (F1), a total of 21 polyps were isolated and all of them produced medusae. No feeding-defective (FD[*1]) strains were observed in F1. In generation 2 (F2), a total of 15 polys were isolated from the cross between B1-7 and B1-1. They all produced medusae. The numbers in the parentheses ([*2]) indicate progeny from the cross between B1-21 and B1-1 (see figure 1). A total of 12 polyps were isolated from the cross and 2 of them were normal and 10 were feeding-deficient. These 2 normal polyps from the cross between B1-21 and B1-1 produced medusae, however, the medusae from the two polyps were feeding-deficient. In generation 5 (F5), a total of 11 polyps were isolated and all of them were FD. Two of the feeding-defective strains produced medusae. All medusae from polyps of F5 were feeding-deficient.

Generation	Polyp		Medusa	
	Nomal	FD*1	Nomal	FD
F1	21	0	21	0
F2	15 (2)*2	0 (10)	15 (0)	0 (2)
F3	13	8	7	6
F4	14	6	7	7
F5	0	11	0	2

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order to continue inbreeding, we performed another cross between B1-1 and B1-7 (female) in generation 2. No polyps from the cross between B1-1 and B1-7 exhibited feeding-deficient phenotype (Fig. 1, Table 1). All the 15 colonies from the cross survived for three months after low-temperature treatment and formed many medusae. In generation 3 (F3), six of the 13 colonies exhibited feeding-deficient phenotype and did not survive. Seven of the 13 colonies survived after low-temperature treatment for three months, and six colonies produced medusae—four females and two males. In generation 4 (F4), seven of the 14 colonies exhibited feeding-deficient phenotype and did not survive. Seven of the 14 colonies survived for three months after low-temperature treatment and seven colonies formed medusae—six females and one male. In generation 5 (F5), all 11 colonies exhibited feeding-deficient phenotype and nine of 11 did not survive. No colonies derived from generation 5 produced any medusae (Fig. 1, Table 1). Therefore inbreeding of the Orinohama lines ended at generation 5.

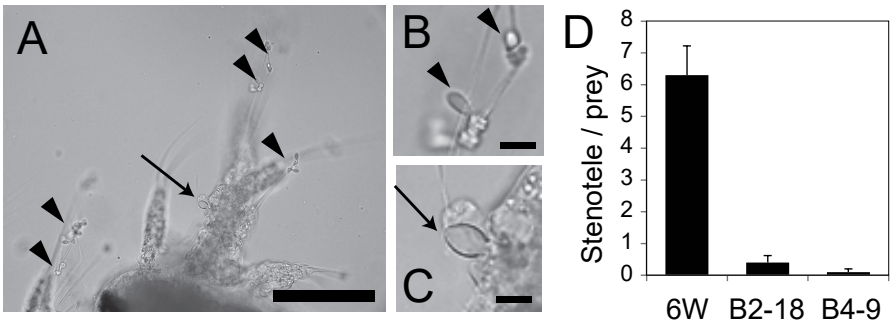


Fig. 4. Stenotele and desmoneme observed on the prey that were captured on the tentacles. **(A)** Stenotele (arrow) and desmonemes (arrowheads) observed on the prey. **(B)** desmonemes (arrowheads). **(C)** Stenotele (arrow). Scale bars: **(A)** = 200 μm; **(B)** = 10 μm; **(C)** = 15 μm. **(D)** Ten brine shrimps captured on the tentacles of each strain (6W, B2-18 or B4-9) were removed immediately after capture and fixed with Carnoy's solution. The stenoteles observed on the prey were then counted.

Feeding-defective strains

Although inbreeding of the Orinohama lines ended at generation 5, we isolated some feeding-defective strains that may shed light on studies of the feeding behavior in jellyfish. These feeding-defective strains could be divided into two categories: (1) feeding-deficient in both polyps and medusae: polyps that showed feeding-deficient phenotype could capture brine shrimps but could not hold, kill, or eat them (Supplementary Movie S4, compare with wild-type polyp in Supplementary Movie S3). Most of these full-time feeding-defective strains could not survive more than a few weeks and we could not maintain these strains. Only one B5-7 strain has managed to survive; however, it would be very difficult to maintain this strain for a long time, (2) feeding-deficiency only in medusae: polyps of some strains like B2-18 and B4-9 can capture and eat brine shrimp normally, but their medusae show feeding-deficient phenotype (Supplementary Movie S2, Fig. 1 and Table 1). We could maintain these part-time defective strains and induce feeding-defective medusae.

Observation of feeding behaviors

In order to identify the cause of feeding deficiency in medusae from the strains, B2-18 and B4-9, we observed the feeding behaviors of these feeding-defective and wild-type medusae. When brine shrimp nauplii touched the tentacles of the wild-type (6W, N3 and B4-1) medusae, they immediately (usually within a few seconds) stopped swimming. The tentacle proximal to the prey then contracts and the prey is hauled into the bell (umbrella) (Supplementary Movie S1). Finally, the prey is put into the mouth. Wild-type medusae consumed more than 90% of the prey that touched their tentacles (Fig. 2B). The medusae of the feeding deficient strains B2-18 and B4-9 could capture brine shrimp nauplii that

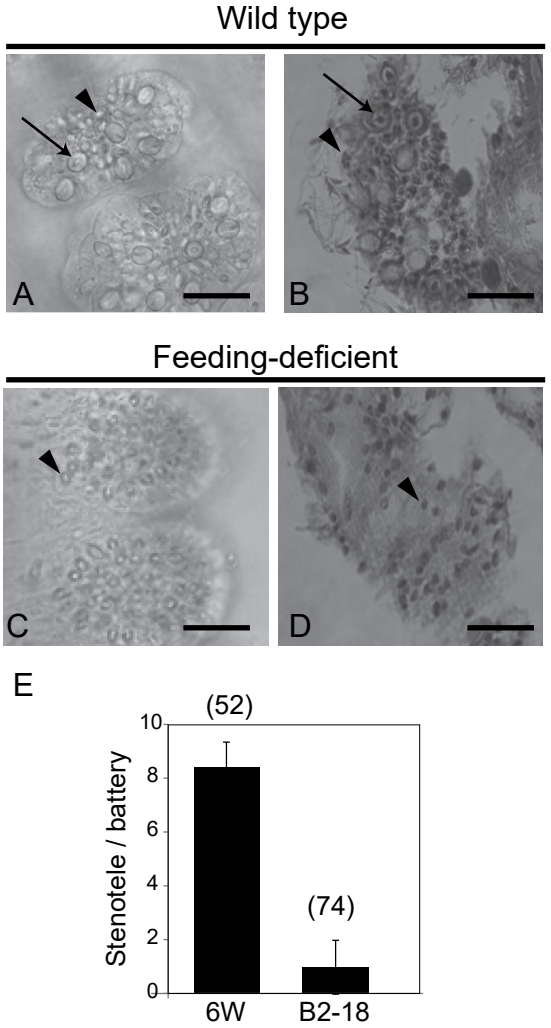


Fig. 5. Stenoteles and desmonemes in the tentacle batteries of wild-type and feeding-defective medusae. **(A–D)** Tentacle battery **(A, B)** wild-type (6W). **(C, D)** Feeding-defective strain (B2-18). Stenotele (arrow) and desmonemes (arrowheads) **(A, C)** Phase contrast, **(B, D)** stained eosin and hematoxylin. Scale bars: **(A–D)** = 25 μm. **(E)** The number of stenoteles in a tentacle battery of wild type medusae (6W) and feeding-defective medusae (B2-18). The total numbers of observed tentacle batteries are indicated in parentheses.

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touched their tentacles and could hold the brine shrimps on their tentacles, but they could not kill them. The brine shrimps that were captured on the feeding-defective tentacles kept swimming (Supplementary Movie S2) and in some cases, they escaped from the tentacles. These feeding-defective medusae ate the prey [25% (B2-18) and 5% (B4-9)] that were captured on their tentacles (Fig. 2B).

Through video analysis of the feeding behaviors of the *Cladonema* medusae, we found that feeding response in a wild-type medusa is described as the following behavioral sequences (see Fig. 2A and Supplementary Movie S1). (1) First, a brine shrimp collides with an outreached tentacle, (2) the prey becomes captured on the tentacle by nematocyst discharge (Fig. 2A, left), (3) the tentacle starts contraction immediately after the prey is immobilized (killed or paralyzed) (Fig. 2A, middle, Supplementary Movie S1), and (4) the contracted tentacle is then bent at its base and is put into the bell, which brings the prey near the mouth (Fig. 2A, right). This sequence of behaviors (1)–(4) occurs as a series of actions, however, there is a latent period before the initiation of tentacle contraction (2) and another latent period before the initiation of tentacle bending (4). We measured these latent periods, namely, the mean times between (2) and (3) (Fig. 3A) and between (3) and (4) (Fig. 3B). For example, a wild-type tentacle (Supplementary Figure S1 A, b in the yellow box) captured prey in 2 sec after the video started, then contracted in 7 sec, and bent in 10 sec. In feeding-defective medusae the early feeding behavioral sequences, (1) and (2), occurred in much the same way as wild-type medusae. However, the tentacle contraction started 2–26 sec (mean 9 sec) after capturing the prey in wild-type while in feeding-defective medusae most of the tentacle which captured the prey did not contract within 60 sec (Fig. 3A). For details on the feeding-defective strains in Fig. 3A, we measured a total of 47 samples of the feeding-defective tentacles and found that 36 of the 47 samples did not contract during the video recorded. One bent in 78 sec, and these 37 samples are showed in >60 (sec), while the others (10 samples) contracted after some delay of 16–54 sec. After contraction, the wild-type tentacles bent within 0–12 sec (mean 5 sec) (Fig. 3B), however, no feeding-defective tentacle bent during the video recorded. We scarcely ever saw tentacle bending in feeding-defective strains in the long-term observation (10–30 min).

Nematocysts

The medusae of the feeding-defective strains could capture and hold the prey on their tentacles but they could not kill them (Fig. 2B, Supplementary Movie S2). From these facts, we deduced that the tentacles of these feeding-defective strains had desmonemes, which are required to capture and to hold prey, but not stenoteles, which are required to kill prey. To investigate this, we recovered 10 brine shrimps immediately after they were captured by a tentacle of wild-type or feeding-defective medusae and then observed the numbers of desmonemes and stenoteles on the brine shrimps (Fig. 4A–D). A lot of desmonemes were observed on the brine shrimps that were recovered from both wild-type and feeding-defective tentacles (Fig. 4A and B, arrowheads). In contrary to the desmonemes, the stenoteles were observed only on the brine shrimps recovered from the wild-type medusae (Fig. 4A and C, arrow) but not from the feeding-defective strains (Fig. 4D). In the hydrozoan jellyfish, tentacles mature functional stenoteles are stored in the tentacle batteries (Denker et al., 2008). Although there seems to be no difference in the morphology of the tentacle batteries between the wild type and feeding-defective strains by the observation with low-magnification microscopy (Supplementary Figure S1, Supplementary Movies S1, and S2), the observations of the batteries through a high-powered microscope showed an obvious difference in the number of stenoteles between wild-type and feeding-defective batteries (Fig. 5A and C). The loss of stenoteles in the feeding-defective tentacle batteries was confirmed by histochemical observations (Fig. 5B and D). There were 8 stenoteles in one wild-type tentacle battery, but 0.97 in one feeding-defective battery of B2-18 (Fig. 5E).

When a brine shrimp swam into a bell of feeding-

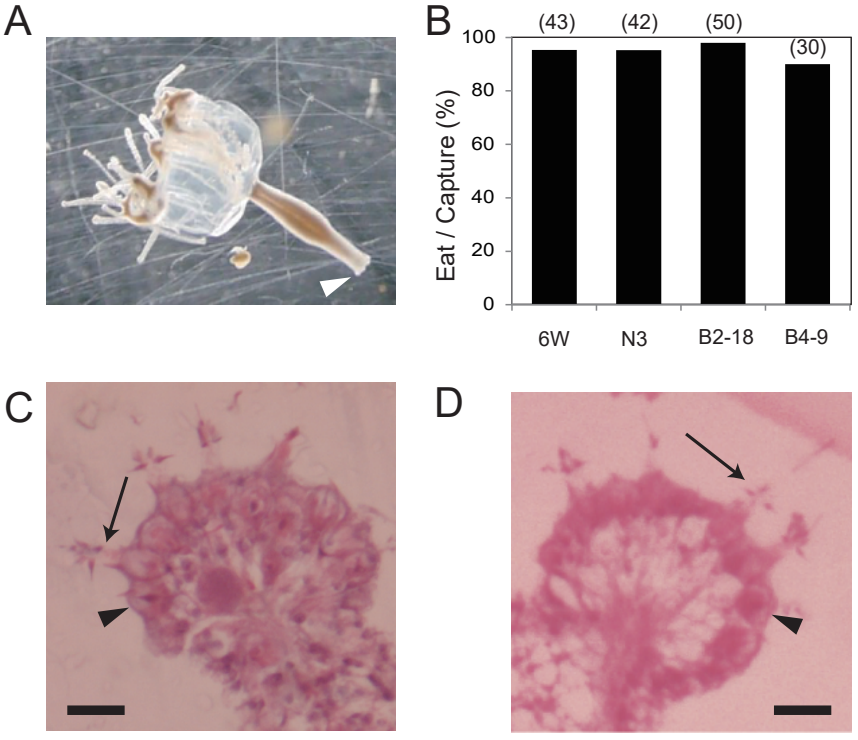


Fig. 6. Stenoteles in the mouth batteries of wild-type and feeding-defective medusae. **(A)** An inside out wild-type medusa (6W). White arrowhead shows oral tentacles. **(B)** The percent of brine shrimps that were engulfed / captured by wild-type medusae (6W and N3) and feeding-defective strains (B2-18 and B4-9). The total numbers of observed brine shrimps are indicated in parentheses. **(C, D)** Oral tentacle, stenotele before discharge (arrowhead) and after discharge (arrow) **(C)** wild type (6W), and **(D)** feeding-defective strain (B2-18). Scale bars: **(A)** = 1 mm; **(C, D)** = 15 μm.

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defective medusa spontaneously without touch on the tentacles and contacted on the battery of the mouth, the prey immediately stopped swimming and was then swallowed. This fact suggests that the stenoteles in the batteries on mouth of feeding-defective strains are functional. To examine whether stenoteles in batteries on the mouth of feeding-defective feeding-defective medusae are functional or not, we turned medusa' s bell inside out (Fig. 6A) to put its mouth out of its bell and gave brine shrimps to the inside out medusae. This inside out medusae of both wild types (6W and N3) and feeding-defective strains (B2-18 and B4-9) could eat almost all (> 90%) the prey that were captured directly by the mouth (Fig. 6B). We confirmed the presence of stenoteles in the batteries on the mouth of wild-type and feeding-defective medusae by hisitochemistrical observation (Fig. 6C and D).

Nematogenesis

As described above, the feeding-defective strains have very few stenoteles in their tentacle batteries, and this loss of stenoteles causes the feeding deficiency. In hydrozoan jellyfish, the ectoderm of the tentacle bulb is a specialized territory of the medusa that functions as a nematocyte production center as described by Denker et al. (Denker et al., 2008). The tentacle bulb is polarized with a clear progression of successive nematoblast stages form and demonstrated a continuous displacement of differentiation nematoblasts towards the tentacle tip. Nematogenesis in *C. pacificum* appears to occur in the same way as in *Clytia hemisphaerica* (Denker et al., 2008, Fig. 7A). To identify the cause of feeding deficiency in the feeding-defective strains, we observed tentacles of wild-type and feeding-defective medusae. We found that the nematoblasts were produced in both the wild-type (Fig. 7A) and the feeding-defective strains (Fig. 7B) tentacles at the maturation area (Fig. 7C). However, few functional mature stenoteles were found in tentacle batteries of feeding-defective strains (Fig. 5E). These facts suggest that the stenoteles are differentiated from stem cells to nematoblasts normally even in feeding-defective tentacles (Fig. 7B), however, nematocytes are not located to the tentacle batteries correctly in the feeding-defective tentacles (Fig. 7C).

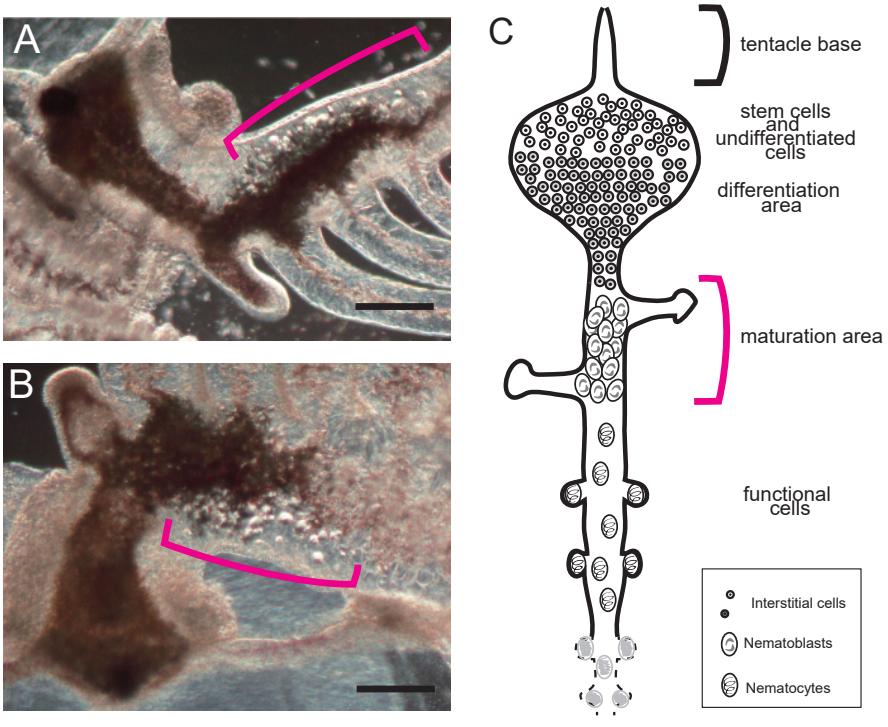


Fig. 7. Nematogenesis in the tentacles of wild-type and feeding-defective medusae. **(A, B)** Nematoblasts in the maturation area (magenta square bracket) in the tentacles of **(A)** wild type (6W) and in **(B)** feeding-defective strain (B2-18). Scale bars: **(A, B)** = 100 μm. **(C)** Diagram illustrating the proximo-distal distribution of nematogenesis stages in the tentacle bulb ectoderm.

DISCUSSION

Inbreeding

The inbreeding of *C. pacificum* of the Orinohama population failed at generation 5 because of the feeding-deficient phenotype (Fig. 1). This phenotype arose at generation 2 and with a high frequency (every polyp from generation 5 was the phenotype) (Table 1). The appearance of the phenotype suggests that many factors may have been involved in the phenotypic expression. These observations suggest that the feeding-deficient phenotype is generally associated with increased homozygosity of the jellyfish genome. This phenotype is an example of inbreeding depression (Charlesworth and Willis, 2009). We do not know why the Orinohama population of *C. pacificum* showed this phenotype. However, this type of inbreeding depression might not be general in *Cladonema* genus because another *Cladonema* species, *C. radiatum*, did not show this phenotype at all in their inbreeding even after generation 6 (Tachibana et al., unpublished observation). We have been analyzing *C. pacificum* genome parental strains of the inbreeding, 6W and N3, and their inbred descendants, B3-9 (male) and B4-1 (female) (Fig. 1). If we could compare the genomes of these strains and feeding-deficient strains, the causative loci might be identified and we could avoid the feeding-deficient phenotype.

Feeding behavior

We isolated the feeding-defective jellyfish strains in the course of inbreeding. These feeding-defective medusae can capture and hold prey on their tentacles but cannot kill them (Fig. 2A and Supplementary Movie 2). This phenotype is caused by the dysfunction of the stenoteles in the tentacles (Fig. 4). The stenoteles penetrate integument of the prey and deliver toxin that causes leaking of body fluid of the prey. In many cnidarians, the chemical signals from the body fluid of the prey evoke feeding behavior (Lindstedt, 1971a). Tentacle contraction in wild-type medusa is initiated immediately after the prey is immobi-

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lized (injured by stenoteles) (Supplementary Movie S1). Taken together, tentacle contraction in the wild-type *Cladonema* medusae appears to be induced by a chemical signal from the body fluid of the prey. However, in the feeding-defective strains, the tentacle contraction hardly occurs. Even when it occurs, the feeding-defective tentacles contract very slowly with a long delay (Fig. 3A and Supplementary Figure S1). It is possible that a very few stenoteles in the feeding-defective tentacles may cause a very little injure to the prey and leak a very small amount of body fluid which might not tentacle contraction or might induce weak and slow tentacle contraction. On the other hand, repeated physical stimulation (by the prey swimming violently) appears to induce tentacle contraction in the feeding-defective strain (Supplementary Movie S2 and Supplementary Figure S1B). It is also possible that tentacle contraction is induced without body fluid (chemical signal). Another latent period is observed between the tentacle contraction (3) and bending (4) in the wild type. In the feeding-defective medusa, the tentacles scarcely ever bend, and even when they bend, the latent period is much longer than that in the wild type (Fig. 3B). Tentacle bending always follows tentacle contraction. The contraction may be prerequisite for the initiation of bending. When strong stimuli, such as *Artemia* homogenates, induce feeding reaction, tentacle bending occurs immediately after contraction (Supplementary Movie S5) in the wild-type and even in feeding-defective strain (Supplementary Movie S6). Furthermore, to test sensitivities of wild type and feeding-deficient medusae to *Artemia* body fluid, we prepared serial two-fold dilution of *Artemia* extract and measure the response to the extracts. This experiment shows that both wild type and feeding-deficient medusae have almost the same sensitivity to the *Artemia* body fluid (Supplementary Figure S2). Although physical stimulus might be able to induce tentacle contraction, it never induces tentacle bending. (Fig. 3B). Similar biphasic feeding response is reported in sea anemone, *Anthopleura elegantissima*, and the two phases of response are controlled by two different chemical signals, asparagine, and GSH (Lindstedt, 1971b). Although it is unknown what triggers tentacle bending in *C. pacificum*, the tentacle shrinks during tentacle contraction and the moving region is the tentacle itself, whereas, in tentacle bending, the contracted tentacle is fixed and the moving region is the base of the tentacle, and not the tentacle itself (Fig. 2A right, Fig. 7A, Supplementary Movie S1). Therefore, bending may be regulated by a different nerve circuit from one is involved in the tentacle contraction. We sought to identify the chemical signals that induce feeding responses in *Cladonema* medusae, however, neither GSH nor some amino acids induced tentacle contraction or bending. To identify the gene(s) involved in the feeding-defective phenotype, deficient of stenotele transfer from tentacle bulb to tentacle battery, genetic and genomic approaches would be required. We have been proceeding with a whole-genome project of *C. pacificum* and assembled a draft genome sequence (N50 = 874 Mbp). Inbred lines, draft genome, and the easiness to maintain render *C. pacificum* a promising experimental animal for understanding the mechanisms regulating feeding-behavior in the cnidarians.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

KT and MM designed and performing research, analyzed data. KT and RD wrote the paper. KT, MM, AM, and RD collected and maintained animals.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs190122)

Supplementary Figure S1 (zs190122.s1.pdf). Analysis of the feeding behaviors in *Cladonema* medusae.

Supplementary Figure S2 (zs190122.s1.pdf). Dose–response curve of the feeding behaviors in *Cladonema* medusae for *Artemia* extracts.

Supplementary Movie S1 (zs190122.s1.mov). Feeding behavior of *C. pacificum* wild-type medusa (6W).

Supplementary Movie S2 (zs190122.s2.mov). Feeding behavior of *C. pacificum* feeding-deficient medusa (B2-18).

Supplementary Movie S3 (zs190122.s3.mp4). Feeding behavior of *C. pacificum* wild-type polyp (6W).

Supplementary Movie S4 (zs190122.s4.mp4). Feeding behavior of *C. pacificum* feeding-defective polyp (B5-7).

Supplementary Movie S5 (zs190122.s5.mov). The response of *C. pacificum* wild-type medusa (6W) to *Artemia* homogenates.

Supplementary Movie S6 (zs190122.s6.mov). The response of *C. pacificum* feeding-deficient medusa (B2-18) to *Artemia* homogenates (see details in zs190122.s1.pdf).

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