Effect of horizontal strong static magnetic field on swimming behavior of *Paramecium caudatum*

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Abstracts

Effect of horizontal strong static magnetic field on swimming behavior of Paramecium *caudatum* was studied by using a superconducting magnet. Around a center of a round vessel, random swimming at 0 T and aligned swimming parallel to the magnetic field (MF) of 8 T were observed. Near a wall of the vessel, however, swimming round and round along the wall at 0 T and aligned swimming of turning at right angles upon collision with the wall, which was remarkable around 1~4 T, were detected. It was experimentally revealed that the former MF-induced parallel swimming at the vessel center was caused physicochemically by the parallel magnetic orientation of the From magnetic field dependence of the extent of the orientation, the cell itself. magnetic susceptibility anisotropy ($\chi - \chi$) was first obtained to be 3.4×10⁻²³ emu cell⁻¹ at 298 K for *Paramecium caudatum*. The orientation of the cell was considered to result from the magnetic orientation of the cell membrane. On the other hand, although mechanisms of the latter swimming near the vessel wall regardless of the absence and presence of the magnetic field are unclear at present, these experimental results indicate that whether the cell exists near the wall alters magnetic field effect on the swimming in the horizontal magnetic field.

Keywords: Strong Magnetic Field; Swimming Behavior; *Paramecium caudatum*; Protists, Susceptibility Anisotropy; Magnetic Orientation

1. Introduction

Effect of a magnetic field, whether it is constant (DC) or oscillatory (AC) in intensity, in biological research fields has long attracted much attention of scientists. One of the reasons might lie in a point of view whether the effect occurs physicochemically or biologically. The studies of the magnetic field effects (MFEs) on organisms carried out till the beginning of 1990s had been already reviewed [1], some of which were imagined to remain uncertain owing to experimentally and instrumentally yielded inaccuracy, and insufficient intensity of the magnetic field used. However, recently developed technique and apparatus enable the scientists to measure even the effect of an extremely small geomagnetic field. Very recently, two groups independently demonstrated the appreciable effects of the geomagnetic field on the movement of a migratory bird [2], a lobster [3], and a sea-turtle [4]. The spin chemistry is now taken notice as a mechanism of the effect on the migratory bird [5, 6]. As the opposite side, on the other hand, the effect of strong magnetic fields of several tesla on organisms is an important subject to be explored since, for instance, a nuclear magnetic resonance imaging (MRI) using such a strong magnetic field is nowadays employed frequently as the technique essential for accurate and right medical inspection. Our group has contributed to the construction of a field of studies, the spin chemistry, through

numerous studies of the MFEs on photochemical reactions in the strong magnetic fields of up to 14 T ([7-9] and references therein). Regardless of the magnetic field intensity, the spin chemistry is now recognized to be one of the core mechanisms for the Besides it, the strong magnetic force and the enhanced magnetic orientation MFEs. are important features in the strong magnetic field, and thereby other MFEs not explained by the spin chemistry can be expected even in organisms at the strong Thus, we initiated to explore the effects of horizontal strong magnetic field. magnetic fields on organisms by using some protists which are well-known to be sensitive to some environmental stimuli such as gravity [10, 11]. In order to remove the influence of microgravity and hypergravity, which are created by vertical strong magnetic force under the gravity, on a protist's nature of sensing gravity (geotaxis), we employed the horizontal magnetic fields and observed protist's horizontal swimming behavior from above a vessel horizontally held. First of all, our group detected two intriguing MFEs in Euglena gracilis (E. gracilis) which contains several tens of chloroplasts inside the cell [12]. One of them was that the swimming behavior was restricted to move perpendicularly to the magnetic field (the MF-induced perpendicular This means that a long axis of the cell orients perpendicularly to the swimming). field (the perpendicular magnetic orientation). Another MFE was that, although each

cell itself kept the perpendicular swimming, the cell distribution in a vessel altered so as to become higher at the side closer to the magnet center at about two hours after the vessel was set in the magnetic gradient generating the strong magnetic force (the Compared with Astasia longa not holding the chloroplasts, positive magnetotaxis). the MF-induced perpendicular swimming was explained by the magnetic orientation of the chloroplasts tightly packed inside *E. gracilis*. Further, the positive magnetotaxis was interpreted by a combination mechanism of the perpendicular magnetic orientation of the cell itself and the inhomogeneous distribution of the diamagnetic chloroplasts As a result, the MFEs of E. gracilis were interpreted inside the cell. physicochemically. In this paper, we present the MFE on Paramecium caudatum (P. *caudatum*) in the *horizontal* strong static magnetic fields. Since P. caudatum has no chloroplasts responsible for the magnetic orientation unlike E. gracilis, the MFE is considered to give a chance to understand the magnetic orientation of the protist in On the other hand, two groups independently reported MFEs on the detail. swimming of a paramecium at a vertical magnetic field where the MFEs should be estimated by taking the influence of gravity into account [13, 14]. However, there was inconsistency between their results that the paramecium swam perpendicularly to the field of 0.68 T [13] in contrast with parallel to the field of 18 T [14]. Since there

might be participation of the vertical strong magnetic force in the gravity in the latter case [14], we had the impression of the necessity of avoiding a use of a vertical magnetic field for *P. caudatum* known to have the geotaxis [11]. In this work, it is shown that *P. caudatum* actually orients and swims parallel to the *horizontal* magnetic field of 8 T. Furthermore, it is revealed that both the position monitoring the swimming in a vessel and the vessel shape affects the MFE.

2. Experimental

A holotrichous ciliate, *P. caudatum*, whose typical size is 200 µm in length and 60 µm in width, consists mainly of a cell membrane and intracellular organs of a macronucleus, a micronucleus, a few thousand of cilia and trichocysts. The trichocyst is docked beneath the cell membrane and released as a needle toward a predator and some stimuli [11]. *P. caudatum* used in this study was cultivated by modifying a standard manner [15, 16]. The cell in the culture was used for the experiment after removing unnecessary precipitates by filtration or after changing the culture into the artificial brine adequate for *P. caudatum*. The cell in the early stationary phase of the growth curve was employed for the experiment.

The horizontal strong static magnetic fields of up to 8 T were afforded by a

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superconducting magnet (Oxford Instruments, SM-1000-11, ϕ 50 mm bore diameter). The horizontal low magnetic fields below 0.8 T were provided by a conventional electromagnet (TOKIN, SEE-9). The vertical strong magnetic fields of 10.7, 12 and 15 T used for comparison were obtained with a superconducting magnet (Japan Superconductor Technology, JASTEC LH15T40, ϕ 40 mm bore diameter). A geomagnetic field, which was normally about 0.05 mT, was treated as 0 T in this study. The inhomogeneity in magnetic field intensity at the each magnetic center, where a vessel containing *P. caudatum* was located, was within 1 % of the field.

A round glass vessel (ϕ = 30 mm) or a rectangular glass vessel (w40 x d10 x h10 mm) containing *P. caudatum* was set inside the horizontal magnetic field equipped with a thermostat maintained at 298 K. The swimming behavior of *P. caudatum* was measured from an upside of the vessel with a CCD camera (OLYMPUS, OH-411) – light source (OLYMPUS, ILK-5) – light guide (OLYMPUS, R100-095-090-50) – display monitor (SONY, EVM-9010R) – digital video cassette recorder (SONY, GV-D1000 NTSC) system. In the case of the vertical magnetic field, the swimming was monitored from a side of the vessel. Every experiment of the measurement was initiated at the same early time in the afternoon to avoid the influence of the circadian rhythm existing in *P. caudatum*. For seeking the magnetic orientation of the cell

which is physicochemically explained by the magnetic susceptibility anisotropy, immobilized *P. caudatum* was prepared by adding

ethylenediamine-N,N,N',N'-tetraacetic acid, disodium salt (EDTA) (0.003 - 0.02 mol/dm³) into the solution containing the living cells in advance. No organic disruption of the cell by the EDTA treatment was confirmed by use of an optical microscope since the treatment simply prevents the signal transduction essential for the swimming by chemically chelating Ca²⁺ as the signal messenger.

3. Results

3.1. Effect of horizontal strong magnetic field on swimming and its magnetic field dependence

[Insert figure 1 about here]

Figure 1 shows snapshots of videos recording the behavior of *P. caudatum* swimming around a center of the vessel in the absence and presence of the *horizontal* strong magnetic field of 8 T. A dark gray ellipse and a white arrow in front of it show a single cell of *P. caudatum* and its swimming direction, respectively. It is clear that

the arrows are in disorder at 0 T (figure 1a) whereas they are almost restricted to orient parallel to the magnetic field of 8 T (figure 1b). We call this effect the magnetic-field (MF)-induced parallel swimming. This parallel swimming direction was independent of the plus/minus sense of the applied magnetic field. Further this swimming appeared immediately after being exposed to the magnetic field, and disappeared without delay when removed from the field. From these results, we recognized that *P*. caudatum was definitely affected by the strong magnetic field so as to swim parallel to the strong magnetic field. In other words, the cell of *P. caudatum* can be said to show the magnetic orientation parallel to the field (the parallel magnetic orientation). Furthermore, it was revealed that the MF-induced parallel swimming speed reduced when the exposure to the strong magnetic field lasted during more than several ten minutes. However, no recovery in the speed was detected even if the cell was removed from the field while the direction of the swimming became in disorder promptly.

[Insert figure 2 about here]

When the horizontal magnetic field increased up to 8 T, the number of the cells

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showing the MF-induced parallel swimming increased. Plots of closed circles in figure 2 display magnetic field dependence (MFD) of a percentage of the cells showing the MF-induced parallel swimming. The percentage was calculated in terms of dividing the number of cells keeping the parallel swimming under the field of view of the microscope by the whole number of cells. After this calculation was repeated by changing the field of view, the percentage was obtained by the average. In the graph, the percentage definitely increases together with increasing the magnetic field. The percentage at 8 T was approximately seven times larger than that at 0 T. Incidentally, whereas the positive magnetotaxis was detected in the case of E. gracilis [12] at the bore position (the magnetic field gradient = $380 \text{ T}^2/\text{m}$) apart from the magnet center, neither positive nor negative magnetotaxis was observed in P. caudatum under the same magnetic field gradient. Furthermore, the pre-treatment of exchanging the culture with the artificial brine afforded no appreciable influence toward the MF-induced parallel swimming and the MFD.

[Insert figure 3 about here]

For comparison, the swimming behaviors of the cell in the vertical strong magnetic

fields of 10.7, 12, and 15 T besides 0 T were shown in figure 3 as well as figure 1 in which the field was *horizontal*. The apparent MF-induced parallel swimming was confirmed even in the three vertical strong magnetic fields. This result was consistent with that of 18 T by Valles's group [14]. The decrease in the swimming speed was also detected during and after the exposure to the vertical magnetic field as well as the horizontal magnetic field.

3.2. Magnetic orientation of immobilized cells

In order to elucidate a mechanism of the MF-induced parallel swimming, we investigated the magnetic orientation of the cell immobilized with EDTA. This is an important experiment because the result leads to reply a question that the MF-induced parallel swimming occurs physicochemically or biologically. Figure 1c exhibits a snapshot obtained from the video recording the orientation of the immobilized *P. caudatum* at 8 T. After the solution containing the immobilized cells was stirred by inclining the vessel compulsorily, the video was recorded continuously until the cells came to a standstill and oriented in the presence of the field of 8 T. Figure 1c is the snapshot being at the standstill, demonstrating that the immobilized cell is arranged parallel to the field. In figure 1c it is found that most of the cells align their long axes

of the ellipse body parallel to the magnetic field.

3.3. Swims at an edge of a round vessel and in a rectangular vessel

[Insert figure 4 about here]

The disordered swimming at 0 T and MF-induced parallel swimming described above were monitored around a center of the round vessel, as shown in figures 4a and 4b. However, when the monitoring position was shifted to an edge of the vessel where the cells collided with a wall, different swimming behavior and its MFE were observed in the absence and presence of the horizontal magnetic field. At 0 T, it was observed that the cells near the vessel wall swam round and round along the wall, as illustrated in By contrast, in the presence of the field, it was detected that most of the figure 4c. cells turned at right angles when they collided with the vessel wall. Concretely speaking, when the cells swimming parallel to the horizontal magnetic field conflicted with the wall, they turned to the direction perpendicular to the magnetic field, as shown in figure 4d. On the contrary, when they first swam perpendicularly to the field, they turned to the direction parallel to the field. The percentage of this MF-induced

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perpendicular swimming, which happened after colliding with the wall of the vessel edge, was plotted against the horizontal magnetic field (see open circles in figure 2). Figure 2 also represented that (i) this MF-induced perpendicular swimming was conspicuous around 1~4 T; (ii) as increasing the field, the MF-induced parallel swimming around the vessel center became predominant at the expense of this MF-induced perpendicular swimming near the wall.

Thus, based on two kinds of swimming behaviors and MFEs depending on the monitoring position in the round vessel, we examined the swimming behavior in a different vessel in shape, a rectangular glass vessel (w40 x d10 x h10 mm) which is very often used in experiments of visible absorption spectroscopy and resembles the vessel (w46 x d10 x h10 mm) of Nakaoka's experiment in size [13]. We monitored the swimming from an upside of the vessel as well as the experiment of the horizontal strong magnetic field already mentioned above. Surprisingly, as a result, it was found that most of the cells anywhere swam parallel to a long axis (40 mm in length) of the rectangular vessel even at 0 T, as illustrated in figure 4e. Moreover, when the vessel containing the cell was set in the conventional electromagnet (~ 0.8 T) in such a way that the long axis of the vessel was parallel or perpendicular to the horizontal magnetic field, neither case showed a change in the swimming behavior, namely, the cell kept the

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parallel swimming along the long axis of the vessel regardless of the magnetic field direction (see figure 4f). The cells in the vessel, whose long axis was set to be parallel to the horizontal magnetic field (figure 4f, left), would have swum in a direction perpendicular to the field (the long axis) if the MFE of *P. caudatum* were the same as that observed by Nakaoka et al. [13], who used a similarly sized rectangular vessel (figure 4g).

4. Discussion

4.1. MF-induced parallel swimming as a consequence of parallel magnetic orientation of P. caudatum

The experiment of the immobilized *P. caudatum* indicates that the MF-induced parallel swimming (figure 1b) observed around a center of the vessel is simply attributed to the physicochemical magnetic orientation of the cell itself as well as the assignment of Nakaoka's and Valles's groups [13, 14]. If this assignment is right, the orientation should be explained by the magnetic susceptibility anisotropy of the cell.

Assuming that the *P. caudatum* is magnetically symmetric along its long axis like a cylinder and possess susceptibilities parallel (χ) and perpendicular (χ) to the axis, the magnetic energy $E(\theta, H)$ per cell at a magnetic field *H* is expressed as

$$E(\theta, H) = -(H^2/2)[\chi_{\perp} + (\chi_{\prime\prime} - \chi_{\perp})\cos^2\theta]$$
(1)

where θ is an angle between the long axis and the magnetic field *H* [17]. In the case of the MF-induced parallel swimming, the angle θ is equal to zero. The magnetic orientation occurs so that the magnetic energy $E(\theta, H)$ becomes minimum. However, the magnetic orientation of the cell holding the magnetic energy $E(\theta, H)$ at temperature *T* is disordered by thermal energy of *T*. According to the Boltzmann statistics, therefore, the probability $P(\theta, H, T)d\theta$ of the cell existing between the angles θ and θ +d θ is written as

$$P(\theta, H, T)d\theta = \frac{\exp[-E(\theta, H)/kT]d\theta}{\int_0^{\pi} \exp[-E(\theta, H)/kT]d\theta}$$
(2)

where *k* is the Boltzmann constant [18]. Here, since the denominator in equation (2) is considered common to all the magnetic fields used, a ratio $R(\theta = 0)$ at $\theta = 0$ of the probability at a magnetic field *H* toward that at 0 T is simplified as

$$R(\theta = 0) = \frac{P(0, H, T)}{P(0, 0, T)} = \exp\left[\frac{H^2}{2kT}(\chi_{//} - \chi_{\perp})\right]$$
(3)

Thus, the logarithmic transformation of both hand sides in equation (3) gives

$$\ln(R(\theta = 0)) = \frac{1}{2kT} (\chi_{//} - \chi_{\perp}) H^{2} = \frac{1}{2kT} \Delta \chi H^{2}$$
(4)

with $\Delta \chi = (\chi - \chi)$. If the experimental result in this work obeys this relation, it reveals that the MF-induced parallel swimming is ascribed to physicochemical

phenomenon of the parallel magnetic orientation due to the magnetic susceptibility anisotropy of the cell.

[Insert figure 5 about here]

Figure 5 is a graph plotted according to equation (4). The plots satisfy the relation within an experimental error, which verifies the parallel magnetic orientation of the cell induced physicochemically , as described above. A straight line superimposed on the plots is the best fitted line acquired by the least-squares method. The anisotropy $\Delta \chi$ of the susceptibility per cell was obtained from the slope to be 3.4×10^{-23} emu cell⁻¹ at the experimental temperature of 298 K. To the best of our knowledge, this is the first evaluation of the anisotropic value per cell of the living *P*. *caudatum*. This value was smaller than values of some substances (benzophenone: 3.0×10^{-20} emu crystal⁻¹; single multiwall carbon nanotube: 6.5×10^{-22} emu nanotube⁻¹; erythrocyte: 8.2×10^{-22} emu cell⁻¹; blood platelet 1.2×10^{-21} emu cell⁻¹) experimentally so far obtained [17-19].

4.2. Origin of parallel magnetic orientation of P. caudatum

We sought an origin of the magnetic orientation of *P. caudatum*. We observed the swimming of P. caudatum parallel to the horizontal magnetic field of 8 T from an upside of the round vessel (figure 4b), while Nakaoka et al. observed the horizontal swimming of P. multimicronucleatum perpendicular to the vertical and horizontal magnetic fields of 0.68 T from a side and an upside of the rectangular vessel, respectively [13] (figure 4g). The definite and important distinction was a direction of the magnetic orientation, namely, the parallel and perpendicular swimmings to the field in our and Nakaoka's results, respectively. Further, Nakaoka et al. also measured parallel magnetic orientations of two principal organs of cilia and trichocysts, of which respective long axes were both parallel to the low field used. Since the cilia grow perpendicularly from the cell surface and the trichocysts are buried maintaining the long axis at right angles to the surface, they led to the conclusion that the perpendicular magnetic orientation of the cell results from the magnetic orientation of Since a side of the cell surface is by far wide in area, the magnetic the two organs. orientation caused by the two organs at the side is more remarkable than in the head and tail. However, this interpretation is inapplicable to our case of the parallel magnetic orientation of P. caudatum. Thus, we examined a cell membrane as a candidate of the origin. It is well-known that the membrane consists of a bi-layer of upright

phospholipids which have long chains of hydrocarbons. Since such a long hydrocarbonaceous chain is found to have a certain magnitude of magnetic susceptibility anisotropy [20], the membrane as an assembly of the upright hydrocarbons should be aligned to the magnetic field. For instance, stearic acid $(CH_3(CH_2)_{16}COOH)$ possesses $\chi = -235.7 \times 10^{-6}$ emu mol⁻¹ and $\chi = -208.2 \times 10^{-6}$ 10^{-6} emu mol⁻¹) [20]. The relationship of $\chi < \chi$ indicates that the membrane comprising many upright stearic acids is arranged parallel to the magnetic field. Therefore, this arrangement of the membrane is proper to explain our observed magnetic orientation of the cell itself parallel to the magnetic field since a side of the non-spherical cell is wider in area than a head and a tail. If we roughly calculate the magnetic susceptibility anisotropy of the membrane based on assumptions that (i) the membrane consists of only stearic acid which has a cylindrical structure and (ii) the cell is also symmetric like a cylinder of 200 μ m in length and 60 μ m in diameter, then it is approximately estimated to be $\Delta \chi = 1.5 \times 10^{-17}$ emu cell⁻¹ by taking account of a diameter of cylindrical stearic acid. This value is considerably larger than that $(\Delta \chi =$ 3.4×10^{-23} emu cell⁻¹) obtained for the cell in this study. However, the difference in the two values seems to be compensated with the anisotropy of cilia and trichocysts. Judging from the direction of the magnetic orientation of cilia and trichocysts measured by Nakaoka et al., the relationship between χ and χ of the two organs is certainly χ

> χ as opposed to χ < χ of stearic acid. Therefore, adding the magnetic orientation of the two organs leads to reduce a value of the susceptibility anisotropy $(\Delta \chi)$, that is, the obtained small value $(\Delta \chi = 3.4 \times 10^{-23} \text{ emu cell}^{-1})$ means an apparent value which results from a total effect due to several substances having independently different susceptibility anisotropies. The smallness of the apparent $\Delta \chi$ value of *P*. *caudatum* might imply that $\Delta \chi$ for the membrane is merely different in the absolute value from a total $\Delta \chi$ for the two organs, though the sign is opposite to each other. In other words, the smallness might suggest that P. caudatum has a tendency of easy alteration of the magnetic orientation (the MF-induced swimming) of the cell by the scanty difference and sign in $\Delta \chi$ of the cell membrane and the combination of cilia and trichocysts. Hence, it might first be said that the difference in the magnetic orientations between us and Nakaoka et al. arises from a difference in a species of paramecium though we refer to an effect of a vessel shape, as mentioned hereafter.

4.3. Dependence of swimming behavior on vessel position and shape for observation In the case of our experiment using *P. caudatum* in a round vessel, we observed two kinds of swimming even at 0T, namely, the random swimming at the vessel center and the swimming around and around along the vessel wall. Further, when a rectangular vessel was used, we detected the aligned swimming along the long axis even at 0 T. These results may indicate that *P. caudatum* has properties to recognize a wall of the vessel and thereafter swim along it. In other words, those strongly suggest that one needs to pay attention to such monitoring position and vessel shape as seeing influence of a magnetic field. In actual fact, we recorded the different MF-induced swimming behavior and the MFD between the center and edge of one round vessel. We explained the mechanism of the MF-induced parallel swimming monitored at the center of the vessel, as already mentioned above. At this stage, however, we can offer no good idea in explanation of mechanisms for both behaviors of swimming along the vessel wall at 0 T and of changing from the swimming at 0 T to turning at right angles upon collision with the wall in the presence of a magnetic field. Nevertheless, it might not be denied that this influence of the vessel besides a species of a paramecium mentioned above is also concerned with the inconsistency between our and Nakaoka's MFEs. Furthermore, the observation of the decrease in the swimming speed during and after the exposure of horizontal or vertical magnetic fields might be concerned with the discrepancy existing between us and Nakaoka et al. M. S. Rosen and A. D. Rosen explained the decrease in the speed of motility may arise from alteration in function of

Ca²⁺ channels induced by the magnetic orientation of the cell membrane [21]. If this is the case, the pre-treatment and cultivation using specifically prepared ionic solution, which were actually carried out in the experiment of Nakaoka et al., are sufficiently predicted to cause the different MFE on the swimming behavior. Experiments for elucidating the mechanism are now under consideration.

5. Conclusion

In this study we revealed the MF-induced parallel swimming of *P. caudatum* around the center of a round vessel results from the magnetic orientation of the cell due to the magnetic susceptibility anisotropy. We proposed the possibility of the cell membrane as the origin of the magnetic orientation by evaluating the susceptibility anisotropy value $\Delta \chi$ of the cell. Furthermore, we measured another swimming behavior and the MFD near the edge of the same round vessel, by which we presented the necessity of strict control over the experimental conditions to compare MFEs.

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Figure Captions

Figure 1

Snapshots of videos recording the behavior of *P. caudatum* around a center of the round vessel in the case of (a) living cells at 0 T; (b) living cells at 8 T; and (c) immobilized cells at 8 T, respectively. All snapshots are taken from an upside of the round vessel (i.e. top view). Original magnification is $\times 20$ in all cases. One dark gray spot corresponds to one single cell. Arrows drawn in (a) and (b) indicate the swimming direction of each living cell.

Figure 2

MFDs of the percentages of *P. caudatum* showing the MF-induced parallel swimming around a center of the round vessel () and the MF-induced perpendicular swimming at an edge of the round vessel (---- ---). The horizontal magnetic field is employed. For the value at 0 T, the cells were counted up, which swam to the same direction with that of the magnetic field when the field was applied.

Figure 3

Snapshots of videos recording the swimming behavior of P. caudatum in the vertical

magnetic fields of 10.7, 12, and 15 T besides 0 T. All snapshots are taken from a side of the vessel (i.e. side view). Original magnification is \times 10 in all cases. One gray spot corresponds to one single cell. Arrows indicate the swimming direction of each living cell.

Figure 4

Illustrations of swimming behaviors in two kinds of vessels and their MFEs. (a)-(f): this study; (g): Nakaoka's study.

Figure 5

A ratio of $\ln(R(\theta=0))$ against a square of the horizontal magnetic field of *H* plotted according to equation (4). The straight line superimposed is the best fitted line toward the observed plots estimated by a least squares method.

Figure 1 Y. Fujiwara et al.



Figure 1 Y. Fujiwara et al.



Figure 3 Y. Fujiwara et al.



Figure 3 Y. Fujiwara et al.



Figure 5 Y. Fujiwara et al.



Figure 5 Y. Fujiwara et al.