Two-state model of *Paramecium bursaria* thigmotaxis

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Abstract. A theoretical framework has been developed for analysis of the interaction between *Paramecium bursaria* and a glass surface. Adhesion to and detachment from a solid substrate were considered in the model as transitions between alternative states in cell behavior: (a) swimming, and (b) motionless (positive thigmotactic) state. According to the model and experimental data, a change in the fraction of swimming cells is described by a negative exponential course. The proposed model allows positive thigmotaxis, generally referred to in the literature simply as thigmotaxis, to be considered as the rate-constant of transition into the motionless state. This approach permits quantitative determination of thigmotaxis, and reveals its dependence on the phase of culture growth and the type of medium surrounding the cells. In the mineral maintenance solution, paramecia from the stationary phase of growth swim more slowly than those in the logarithmic phase of growth, and show enhanced thigmotaxis. However, a general relationship between thigmotaxis and swimming speed was not established.

Key words. *Paramecium bursaria*; thigmotaxis; swimming speed; culture growth phase.

The motile behavior of *Paramecium* depends on the action of several thousands of cilia. Under normal ambient conditions, these beat in a coordinated way obliquely backwards[1-3], so that the cell swims forwards along a left-hand spiral line. The forward movement may undergo a sudden perturbation if the cell encounters a stimulus of sufficient intensity to evoke a change in the direction of locomotion. Depending on the stimulating factor, paramecia may swim in a different mode[4,5]. In media providing favorable living conditions, such as an abundance of bacterial food[6], optimum pH and ionic composition[7-11], optimum temperature[2,14,15] or light conditions[14-20], paramecia can slow their forward movement or can even stop it for some time.

Paramecium may respond by acceleration of its forward swimming when a mechanical stimulus acts on the posterior end of the cell. Collision of the swimming cell with a solid obstacle, (i.e. mechanical stimulation of its anterior part) evokes one of two responses: a) transient reversal of swimming, a typical 'avoiding reaction'[2,21,22], or b) cessation of swimming due to continued contact with the obstacle for some time[1,23]. Therefore, interaction between ciliates and a solid substratum can be considered in terms of thigmotaxis. In this context, the 'avoiding reaction' is an example of negative thigmotaxis, and cessation of swimming and establishment of contact with the substratum is a positive thigmotactic response. Being in contact with a solid surface, paramecia can: 1) creep along it, or 2) stay motionless. The former was observed mainly in *Paramecium bursaria* cells on a glass surface[24]. The latter is usually referred to as an adhesion or attachment because a thigmotactic (mechanical) stimulus is often associated with additional stimuli resulting from the particular chemical and/or physical properties of the substratum[25]. For example, the attachment of ciliates on polystyrene involves hydrophobic interactions, and takes place during their first contact with the surface. Cells may remain attached even for a few hours. This behavior was observed in various species of *Paramecium*[24] and is closely connected with mating reactivity[23-29]. The attachment occurs only at the tips of the ventral cilia, to which this activity is restricted[26-30].

In this paper we show that interaction between *Paramecium bursaria* cells and a glass surface is satisfactorily approximated by a simple two-state model with mass-action kinetics. It allows us to define positive thigmotaxis as a rate-constant establishing the contact with solid surface, to quantify thigmotaxis.

Materials and methods

Chlorella-containing *Paramecium bursaria* (strain F 9) kindly provided by Dr Watanabe (Tohuku University) was used. Cells were cultured in darkness at 21 ± 2 °C in Scottish grass infusion medium inoculated with *Klebsiella aerogenes* one day before use. The cultures were fed for 4 successive days by adding an equal volume of fresh medium once a day until the logarithmic phase of growth was reached. Within 7 days after the last food supply, the cultures reached the stationary phase. Since the cultures of *P. bursaria* used in the experiments were derived from stock cultures maintained in constant darkness for several months, the number of algae per cell was reduced to 50–150.

The swimming speed and thigmotaxis of paramecia were examined in the following media. a) Culture medium from the growth phase of *Paramecium bursaria* under investigation, devoid of particulate material. This was obtained by two-step centrifugation: 2500 × g for 2 min to harvest the paramecia, and then 13000 × g for 30 min to remove bacteria and particulate detritus. b) Mineral maintenance solution consisting of 1 mM KCl, 1 mM $CaCl_2$ and 5 mM Tris-HCl buffer pH 7.2, also free of particulate material[31].

The cells of *P. bursaria* of the phase of growth under investigation were concentrated and transferred into the test media by means of galvanotaxis (0.79 V cm^{-1}) and equilibrated in test tubes for 1 h.

To measure the swimming speed of paramecia and thigmotaxis a modification of the method of Dryl[7, 32] was used. Samples of about 200 cells in a given experimental medium were poured into an experimental glass chamber (21 mm × 29 mm) bordered by 150-µm-thick, transparent, self-adhesive, inert plastic tape (Con-Tact; Storeys, England) and covered with a cover-glass of appropriate size. At 5, 15, 30, 45 and 60 min, the test chamber was photographed three times, with an exposure of 3 s and 0.5–1-s intervals between consecutive pictures. The chamber was continuously illuminated by two 6-V incandescent bulbs with $CuSO_4$ heat-absorbing filters (1400 lx). The illumination had been tested in preliminary experiments and found suitable. In the photographs, the cells settled on the glass surface appear as dots, while those swimming freely appear as lines of various lengths. By measuring the length of 100–200 cell tracks in three consecutive pictures, the mean speed of cells for each data point was assayed. Each assay was performed 3–6 times. The entire experimental procedure was performed at temperature 21 ± 2 °C.

Results

Paramecium bursaria cells introduced into medium enclosed between the two parallel glass surfaces of the experimental chamber slow down their swimming speed, and adhere transiently to the glass surface. Therefore, it is plausible to assume that each cell of the population remains in one of two states at a given time: a) swimming or b) motionless (positive thigmotactic). Hereafter, 'positive thigmotaxis' will be referred to as a 'thigmotaxis'. Such behavior of ciliates is schematically presented by the following cycle:

$$\begin{tikzcd} x \arrow[r, bend left, "v_1"] & y \arrow[l, bend left, "v_2"] \end{tikzcd} \; ; 0 \leq x,y \leq 1 \qquad \{1\}$$

where: x and y are the swimming and motionless fractions of the cell population, respectively; v_1 and v_2 are the rates of transition which close this cycle.

Assuming: (a) that the number of investigated cells is constant, i.e., $y = 1 - x$ (b) mass-action kinetics, i.e., $v_1 = k_1 x$ and $v_2 = k_2 (1 - x)$, we have the balanced equation for scheme {1}.

$$\frac{dx}{dt} = k_2 (1 - x) - k_1 x \qquad \{2\}$$

This allows determination of the function:

$$y(t) = \frac{k_1}{k_1 + k_2} \{1 - \exp[-(k_1 + k_2) t]\} \qquad \{3\}$$

which describes changes of the motionless fraction of the cell-population over time.

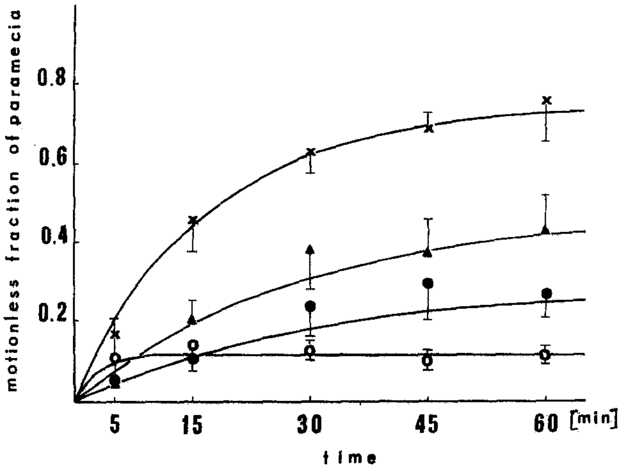

Figure 1. Thigmotaxis of *Paramecium bursaria* cells. The curves which approximate experimental data (±SE) are drawn in accordance with eq. 3. The values of rate constants k_1 and k_2 which determine the curves are marked by appropriate markers in fig. 2. x – paramecia of stationary phase in mineral maintenance solution (n = 5798), ▲ – paramecia of stationary phase in culture medium (n = 6796), ● – paramecia of logarithmic phase in mineral maintenance solution (n = 2639), o – paramecia of logarithmic phase in culture medium (n = 2284). Abscissa: fraction of paramecia remaining motionless, ordinate: time in minutes.

The theoretical considerations presented above were verified in four different experiments. The phase of culture growth and the type of medium seem to be the main factors affecting thigmotaxis. Therefore, thigmotaxis of *P. bursaria* cells from the logarithmic or stationary phase was examined in two test solutions: (1) mineral maintenance solution, and (2) the culture medium. Data obtained in these experiments are presented in figure 1. The function y (t) {Eq. 3} is within the ranges of error deviation points for each of the sets of data presented in figure 1. Therefore, the model is applicable for analysis of the interaction between ciliates and a solid surface. It allows us to define thigmotaxis by the rate constant k_1, while the rate constant k_2 characterizes detachment from the substratum.

An advantage of the proposed model is its applicability for analysis of the behavior of ciliates with respect of thigmotaxis and detachment, separately. These two processes determine both the level of the steady-state $\left(\alpha = \frac{k_1}{k_1 + k_2}\right)$ and the time for attaining its half value $\left(t_{1/2} = \frac{\ln 2}{k_1 + k_2}\right)$. The table shows ranges of variability of thigmotaxis (k_1) and detachment (k_2) under the experimental conditions considered. These results are completed with values of α and $t_{1/2}$.

Another approach is the consideration of thigmotaxis and detachment as an ordered pair (k_1, k_2) which defines a point in the plane enclosed by the k_1 and k_2 coordinates. Figure 2 shows four separate sets of (k_1, k_2). Any pair of k_1, k_2 from these sets determines the function which approximates to the corresponding experimental

The range of variability of parameters describing *P. bursaria* thigmotaxis. k_1: rate constant of transition to motionless state, k_2: rate constant of detachment, $t_{1/2}$: half time of thigmotaxis equilibrium attained by *P. bursaria* cells, α: equilibrium level. The range of variability of parameters was estimated by computer scanning of k_1 and k_2 values according to the criterion given in the legend to figure 2.

	Culture growth phase			
	Logarithmic		Stationary	
Medium	Culture	Mineral	Culture	Mineral
k_1 [x10^{-4}s^{-1}]	2.8-*	1.2- 2.3	1.8 - 3.3	5.5 - 8.1
k_2 [x10^{-4}s^{-1}]	20.0-*	0.2- 8.3	0 - 5.8	0.5 - 4.0
$t_{1/2}$ [min]	*-5	11.0-82.0	13.0 -64.0	10.0 -19.0
α	0.11-0.13	0.22- 0.86	0.36- 1.0	0.67- 0.91

* Values of experimental data which could not be estimated.

data shown in figure 1. The pairs (k_1, k_2) which define the curves drawn in figure 1 are marked in figure 2. The set of k_1, k_2 for cells from the logarithmic phase of growth in culture medium is not closed, because the dynamic equilibrium between the number of swimming and motionless cells was attained before experimental determination of the swimming fraction of cells could be recorded. In spite of this, figure 2 shows that the sets of k_1 and k_2 are distinctly separated. This leads to the conclusion that both the phase of growth and the nature of the surrounding medium have noticeable and measurable influence not only on thigmotaxis but also on the spontaneous detachment of ciliates from the glass surface. However, we did not find a general relationship between thigmotaxis of *P. bursaria* cells and their swimming speed. Figure 3 shows that the swimming speed decreases markedly during the first 30 min after introducing ciliates into the test chamber, then attains an almost stable level. Such behavior is most pronounced in paramecia maintained in a mineral solution. In this medium, the higher level of swimming speed of paramecia is associated with lower thigmotaxis (compare figs 2 and 3). On the other hand, similar ranges of variability of thigmotaxis are observed for paramecia which swim at evidently different speeds. The latter is observed for cells in the logarithmic phase of growth in mineral maintenance solution and in the stationary phase in culture medium.

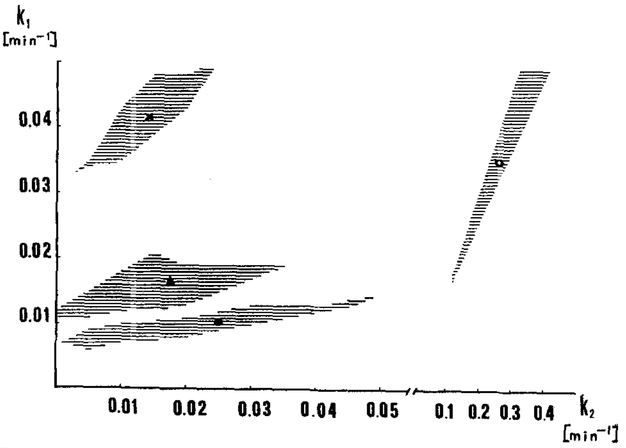

Figure 2. Values of the rate constants, k_1 and k_2, which determine the curves eq. 3 approximating the kinetics of thigmotaxis of *Paramecium bursaria* cells. Dashed areas mark the sets of k_1 and k_2 values which determine curves passing along the all errors range (fitting criterion) of a given set of experimental data shown in fig. 1. Marked pairs of k_1 and k_2 in the sets define curves shown in fig. 1. Other details are described in the legend to fig. 1.

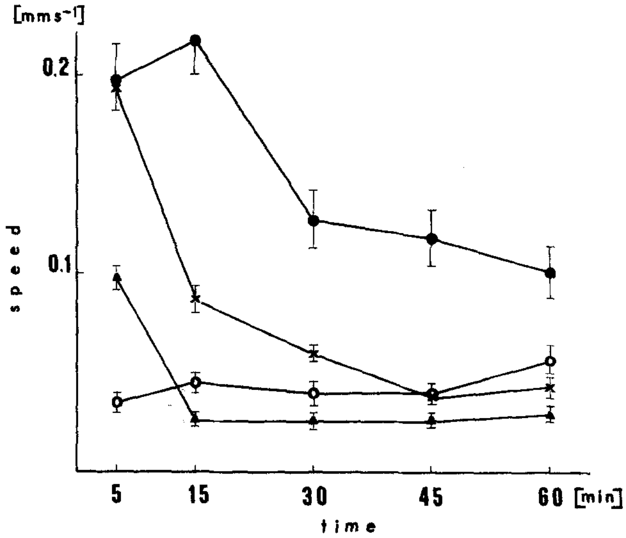

Figure 3. Mean values (±SE) of swimming speed of *Paramecium bursaria*. x – paramecia of stationary phase in mineral maintenance solution (n = 935), ▲ – paramecia of stationary phase in culture medium (n = 774), ● – paramecia of logarithmic phase in mineral maintenance solution (n = 561), o – paramecia of logarithmic phase in culture medium (n = 305). Paramecia were continuously illuminated (1400 lx). Abscissa: swimming speed expressed in mm s^{-1}. Ordinate: time after immersion of cells in solution in experimental chamber.

Discussion

When *Paramecium bursaria* cells are placed on slides for microscopy, or in the experimental glass chamber used in this study, their swimming speed decreases and eventually locomotion ceases. Analysis of their behavior shows that after a period of straight forward swimming an increasing number of cells began to spin, loop, circle and gyrate as the result of ciliary reversal, as was found in other paramecia[4,5,11,15,21]. These changes in locomotion are believed to be evoked by contacts of cells with the substratum which causes an alteration of ciliary beating and general swimming mode. Presumably, the surface receptor sites produce stimuli which pass into the cell of the membrane and act by a change in the electrical properties[2,33-36]. However, *P. bursaria* enclosed between two parallel glass surfaces most frequently touches the substrate with the cilia covering the central parts of the ciliate body, while collisions leading to an 'avoiding reaction'[21] are rather rare. Mechnical perturbation of the somatic cell membrane mediated by cilia[2,37] may promote Ca^{2+}-influx through 'stretch-sensitive channels'[38] which results in hyperpolarization and depolarization of the cell. Frequent collisions may lead to habituation of ciliary beating, as happens with the contractility of

Spirostomum exposed to mechanical stimulation[39]. In the experiments presented in this paper, after about 15–30 min most cells became sluggish, and some of them adhered to the glass surface for either a few seconds or even a dozen minutes. Motile cilia became motionless, and effective beating was localized mainly in the buccal cavity[6].

As mentioned in the introduction, thigmotaxis as a result of mechanical stimulus is often associated with additional stimuli evoking attachment. In order to exclude the possible contribution of mating reactivity of *Paramecium bursaria*, the cells used in the present study were grown in continuous darkness, which depresses mating reactivity[40,41]. These culture conditions result in the reduction of the number of symbiotic *Chlorella* to about 100 per *Paramecium* cell, and the establishment of a balance of step-up, step-down photobehavior responses at the lowest level[11,16,41], in spite of the illumination of cells for photographic recording of swimming speed.

The evidence presented in this paper shows that the frequency of *Paramecium bursaria* cells adhering to the glass surface as a result of thigmotaxis can be quantified. Thigmotaxis, defined as a rate constant k_1 in the elementary model presented in this paper, depends on the culture growth phase from which the paramecia used in the experiments originated, and on the nature of the medium. It is possible that the higher adhesiveness of ciliates in the stationary phase reflects the alteration in lipid content which takes place in the cell membrane during successive culture growth phases. In *P. aurelia* and *P. multimicronucleatum*, the neutral and polar lipids increase on the inoculation of stationary phase cells into new culture medium and afterwards decrease, reaching almost the initial level during 5–7 days. The relative increase of polar lipid fractions is noted after the 5th day in axenic medium[42–45]. However, ciliary lipid composition changes little with growth phase in bacterized cultures[46]. Nevertheless, this may be connected with the enhancement of hydrophobic interaction intensity between the ciliary membrane and the substrate, increasing mating receptivity[47] and the ability to attach to polystyrene surfaces[24,26], as well as thigmotaxis of *P. bursaria*, since this property evidently increases with the culture age of *P. tetraurelia*[48].

The lack of a clear relationship between swimming speed of ciliates and their thigmotaxis suggests that the ciliary activity plays a minor role in establishing the thigmotactic contact of *P. bursaria* cells with the glass surface.

In conclusion, the evidence presented in this paper shows that the frequency of *Paramecium bursaria* cells adhering to the glass surface as a result of thigmotaxis, depends on the culture growth phase from which paramecia used in the experiments originated, and the type of medium surrounding the cells. The theoretical approach developed in this study permitted a satisfactory approximation of the interaction of *Paramecium bursaria* cells with the glass surface by a simple two-state model with mass-action kinetics.

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