CAMBRIDGE
Cambridge University Press
0521820529 - Gravity and the Behavior of Unicellular Organisms
Donat-Peter Hader, Ruth Hemmersbach and Michael Lebert
Excerpt
More information

1

Introduction

1.1 Historical background

The phenomenon that some free-swimming unicellular organisms tend to swim to the top of a tube and gather there – independent of whether the tube is open or closed – has been observed more than 100 years ago. This behavior was termed **geotaxis** (orientation with respect to the gravity vector of the Earth) – negative geotaxis if the organisms orient upward and positive geotaxis if they swim downward (cf. Section 1.2). Nowadays, this term has been replaced by **gravitaxis**. Many early and detailed studies between 1880–1920 provided descriptive observations limited by optical and analytical means. This led to the establishment of various hypotheses that have been reviewed by different authors (Bean, 1984; Davenport, 1908; Dryl, 1974; Haupt, 1962b; Hemmersbach et al., 1999b; Jennings, 1906; Kuznicki, 1968; Machemer & Bräucker, 1992).

The results were rather conflicting and led to controversial interpretations. While Stahl (1880) stated that *Euglena* and *Chlamydomonas* do not orient with respect to gravity, Schwarz (1884) concluded from his observations that *Euglena* moves upward by an active orientational movement and is not passively driven (e.g., by currents in the water or attracted by oxygen at the surface). He found that the force of gravity could be replaced by centrifugal force and that *Euglena* could move upward against forces of up to $8.5 \times g$. The author also concluded that *Euglena* belongs to the negative geotactic organisms. Aderhold (1888) stressed that positive aerotaxis is the major reason for the upward movement of *Euglena*. The interaction of light and gravity on the orientation of *Euglena* was investigated by Wager (1911): “If light is strong, gravity may play little part in controlling the movements; if the light is weak or absent, gravity appears to be the sole determining factor.” He determined the mean specific weight of a *Euglena* cell with 1.016 g cm^{-3}. Massart (1891) investigated different species (bacteria and ciliates) and

Cambridge University Press
0521820529 - Gravity and the Behavior of Unicellular Organisms
Donat-Peter Hader, Ruth Hemmersbach and Michael Lebert
Excerpt
More information

found that they exhibited gravitaxis; the direction of gravitaxis appeared species-specific and, in the case of the flagellate *Chromulina*, temperature-dependent.

Gerhardt (1913) investigated the gravitactic behavior of *Closterium* (Desmids). Distribution and movement direction of these algae on a glass plate within a closed and completely water-filled tube revealed that, after one night in darkness, most of the organisms had moved upward on vertical (slightly meandering) paths, noted by the colored slime threads left behind. To prove whether crystals within the cells were involved in the perception of gravity, he proposed separating these crystals from the cells. Although this was not possible mechanically, he proposed cultivating them in a calcium-free medium, which should have at least reduced the number of crystals. Gerhardt also described the ecological suitability of gravitaxis, because it helps an organism to orient in darkness (e.g., after being buried in the mud). There are methods now available in gravitational biology, such as destruction of the gravisensor by means of a laser beam [e.g., in the case of the ciliate *Loxodes* (cf. Chapters 2 and 4)] or reducing the number of statoliths by, for example, cultivation of *Chara* rhizoids in an artificial barium-free medium (Kiss, 1994).

Moore (1903) stated that the sign of geotaxis in *Paramecium* depended on feeding status and temperature. Sufficient food and elevated temperature favor negative gravitaxis, whereas the cells show positive gravitaxis under "unfavorable conditions, such as lack of food or ice formation on the surface of the water column."

Although gravitaxis is rather easy to observe, an additional gravity-induced response of swimming microorganisms remained undiscovered for a long time. Dembowski (1929b) stated no difference between upward and downward swimming velocities of *Paramecium* cells. This phenomenon could be analyzed in detail by means of computer-controlled image analysis and automatic measurement of high numbers of cell tracks (cf. Chapter 3). This led to the identification of the phenomenon **gravikinesis** – a direction-dependent kinetic response to compensate at least part of the cell's sedimentation rate (cf. Section 1.2.1). The models for gravity perception are discussed in later chapters.

Possible mechanisms leading to gravitaxis have been discussed since the end of the nineteenth century. Different hypotheses were established either proposing pure physical or physiological ones (for reviews, see Barlow, 1995; Bean, 1984; Hemmersbach et al., 1999b; Machemer & Bräucker, 1992; Roberts, 1970). A physical mechanism assumes a passive alignment of the cell (e.g., caused by the heavier posterior cell end). In contrast, the existence of a physiological receptor is postulated for the detection of deviations from the gravity vector, thus initiating a sequence of sensory transduction events that finally result in an active course correction [e.g., controlling the ciliary/flagellary beat pattern (cf. Chapter 9)]. There is no fundamental morphological difference between cilia and flagella (cf. Section 4.1.1), but they are semantically differentiated between the two taxonomic groups.

1.2 Definitions

Microorganisms, as well as multicellular plants and animals, respond to environmental stimuli by a multitude of responses. There have been several attempts

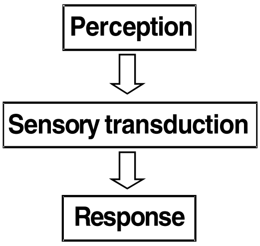

Figure 1.1. Signal transduction chain in living organisms that respond to environmental stimuli (for details, see text).

to develop a unified system of definitions for these responses. Unfortunately, there are two main reasons that have, up to now, prevented generally accepted definitions. One is due to our incomplete knowledge and understanding of the underlying mechanisms. In these cases, we need to wait for a detailed analysis of the receptor and the signal transduction chain resulting in the ultimate motor response to classify the reaction correctly. The second problem stems from the nonuniform usage of terms by different groups and researchers, and noncompliance with definitions already established in the literature.

A true behavioral response of an organism to an environmental stimulus requires the presence of several components. First, the organism needs to possess an appropriate receptor for the physical or chemical nature of the stimulus. This is followed by a signal transduction chain that is often regarded as a "black box," since the individual elements are often not known (Fig. 1.1). Usually, there is a transformation of the energy of the stimulus (e.g., light, gravitational field, or chemical gradient) into a different form of energy (conformation change of a molecule, electric gradient, biochemical reaction) often accompanied by an amplification of the signal. The internal signal is relayed to the actuator (e.g., flagellum or cilium), which may be located in the same cell or in a different one in multicellular organisms, thus generating the visible response. For instance, the gravireceptor for gravitropism in higher plant roots is located in statocytes (statoliths containing cells) organized in a tissue called **statenchym** located in the root columella, while the growth response occurs in the elongation zone of the root several millimeters above the root tip. Amplification can be achieved by gating ion fluxes across a membrane (Nowycky & Thomas, 2002) or a cascade of enzymatic reactions as in vertebrate and invertebrate vision (Müller & Kaupp, 1998; Nagy & Stieve, 1995). This definition of the responses to environmental stimuli excludes passive orientation of organisms by external forces, such as magnetic or electric field lines or acceleration, etc. For instance, magnetic orientation of bacteria is not a taxis in the strict sense, because the cells are passively aligned by the Earth's magnetic field lines due to the presence of small magnetic particles within the cells.

We will attempt to summarize the definitions for responses of organisms to external stimuli, mainly responses to gravity, accepted by most researchers. We will also describe aberrant usage by some groups. We will divide the discussion into one part on motile microorganisms and a second part on higher plants. We will consider multicellular animals only in passing.

For the description of motile behavior in microorganisms, the distinction between plants and animals has proven useless because the behavior can be classified for both kingdoms and the boundaries between them are flexible. One typical example is the taxon of Euglenophytes, which has been claimed by both zoologists and botanists to belong to the animal and plant kingdoms, respectively. The botanists have pointed out that many of the members in this group are photosynthetic – having true chloroplasts – and consequently are plants. In contrast, the zoologists pointed out that they do not have a typical plant cell wall and possess cellular features often found in animals. In this group, there are a number of heterotrophic organisms that lack chloroplasts. Even the same species can be photosynthetic or lose its chloroplasts and live heterotrophically. Based on these considerations, the term "protists," first used by Verworn, may be appropriate to describe these microorganisms (Verworn, 1889b). We will use the term microorganisms in a loose sense for small unicellular and multicellular organisms, including prokaryotes and eukaryotes (i.e., bacteria, cyanobacteria, protists, small algae, and fungi). This usage makes it difficult to draw a line between a larger multicellular microorganism and a small animal or lower plant. But, this distinction may be irrelevant for the current discussion.

1.2.1 Responses of motile microorganisms to environmental stimuli

Motile microorganisms can be powered by different mechanisms, including swimming by flagella or cilia (Melkonian, 1992) or crawling along surfaces by means of membranella or cirri; they can glide, which is described as sliding, twitching, bending, jerking, or ameboid movement (Häder & Hoiczyk, 1992). They can orient the direction of their movement with respect to the source of an external stimulus. This behavior is called **taxis**. The direction of movement can be toward the source of the stimulus (**positive taxis**) or away from it (**negative taxis**). It can also be at an angle with respect to the stimulus direction (**transverse** or **diataxis**), resulting in a bimodal splitting of a population of microorganisms. In any case, the orientation is with respect to a vectorial stimulus, and the cells respond continuously by eliciting course corrections if they are offset from the chosen direction by mechanical forces or random deviations.

The organisms can respond to a multitude of environmental stimuli that is indicated as a prefix to the appropriate term. The directional response to light is called **phototaxis** (cf. Section 7.2). Cells move toward the light source (*positive phototaxis*), away from it (*negative phototaxis*), or at a certain angle (*dia-* or *transverse phototaxis*). The response to a source of a chemical is called **chemotaxis** (cf. Section 7.3). This can be an attractant such as a source of food (e.g., sugar) or a sexual pheromone or a repellent (e.g., phenol). One terminological problem is that the organism cannot perceive the location of the source of the chemical. While a microoganism can detect the location of a light source and move toward it, there is no way of knowing where the source of the chemical is. Therefore, the organisms are limited to a random walk that is biased by a chemical gradient. An organism will move in a randomly chosen direction and continue on this path if it detects an increase over time in the concentration of the (attractant) chemical. It will undergo either organized course corrections or random reorientations if it is going in the wrong direction and senses a decrease in the attractant concentration (cf. Section 7.3). The opposite behavior is observed for repellents. The term **chemotaxis** can be specified by the chemical the organism is responding to, such as in the case of oxytaxis or aerotaxis when the organism orients its swimming path with respect to an oxygen gradient within the water column.

Likewise, the response to a thermal gradient is called **thermotaxis** (cf. Section 7.4), which can be positive (toward the warmer side of the gradient) or negative (toward the cooler side). Some organisms follow the direction of electrical field lines (**galvanotaxis**), even though this behavior may be not a natural phenomenon and induced by a secondary effect (cf. Section 7.6). Bacteria, but also many eukaryotic organisms, have been found to orient with respect to the magnetic field lines of the Earth (**magnetotaxis**). Although it is obvious that magnetotactic orientation is advantageous for higher animals – such as migratory birds – this behavior is somewhat surprising for motile microorganisms: Why would a small cell with a limited swimming range navigate due North or South. The ecological advantage may not be found in the directional movement with respect to the magnetic poles of the Earth, but rather in a mechanism to adjust the vertical position in the water column (cf. Sections 6.4 and 7.5).

The directional orientation with respect to a gravitational field is called **gravitaxis**. In the earlier literature, it was termed **geotaxis**. However, since it is not a specific response of organisms on Earth (greek: *geia*), but can be elicited by any kind of acceleration (e.g., on other celestial bodies or by centrifugation), this term was replaced by the wider term gravitaxis. There is a long and still ongoing discussion among researchers whether the orientation of a given microorganism is due to an active physiological response, including active reorientation (which would be regarded as a true gravitaxis) or by a passive alignment of the cell in the water column, because it might be tail heavy and passively adjusted by the gravitational field (cf. Section 1.1 and Chapter 9). Further details for various organism groups are given in later chapters.

A taxis is a vectorial, long-term response to a given stimulus direction; therefore, the definition of a minimal exposure time is difficult. Usually, an organism orients itself to the stimulus as long as this is present. In contrast, a **phobic response** is a transient reaction to a change in the stimulus strength independent of the direction of the stimulus source (Diehn et al., 1977). This can be best exemplified by the photophobic response of motile microorganisms. When a swimming microorganism experiences a sudden change in light intensity, it responds with a transient behavioral program which, depending on the species, can be a sudden stop, a change in direction, a reversal of movement, or a more complicated pattern of responses. The direction of the response is independent of the stimulus direction; it is irrelevant whether the actinic (stimulating) light beam impinges from above or below or from the side. Thus, the eliciting stimulus can be described as dI/dt, indicating that the change in light intensity has to occur fast. The cells will not respond to slow changes (e.g., during sunrise or sunset). The

Cambridge University Press
0521820529 - Gravity and the Behavior of Unicellular Organisms
Donat-Peter Hader, Ruth Hemmersbach and Michael Lebert
Excerpt
More information

duration of the response is also a built-in property of the behavioral response of the organism (Doughty, 1991). After the transient reaction, the organism resumes its normal swimming behavior. The photophobic response can be elicited by a sudden increase in the light intensity (step-up photophobic response) or a sudden decrease (step-down photophobic reaction). Both reactions can be observed in the same organism, but at different absolute irradiances (Doughty, 1993; Doughty & Diehn, 1984). Similar phobic responses can be induced by changes in other stimulus qualities and are consequently defined, for example, as chemophobic, mechanophobic, thermophobic, etc., reaction. Graviphobic reactions are not easily observed, because the gravitational field on the Earth is rather constant, but sudden acceleration or deceleration may induce graviphobic responses in motile microorganisms (Machemer, 1998).

The third type of motile responses in microorganisms is called **kinesis**. According to the classical definition (Diehn et al., 1977), this reaction is a steady-state dependence of the movement velocity on the stimulus intensity. This response is strictly independent of the stimulus direction. **Photokinesis** describes the dependence of the swimming speed on the light intensity, compared with that in the dark control. If a cell swims faster at a certain irradiance than in the dark, the behavior is described as positive photokinesis; if it moves slower, this is defined as negative photokinesis. The organism may become motionless in darkness (termed **Dunkelstarre** by the earlier German authors) or immotile in bright light (**Lichtstarre**). Kinesis has also been found to be induced by other stimuli qualities (Dinallo et al., 1982; van Houten et al., 1982; Zhulin & Armitage, 1993).

In some ciliates, a kinetic behavior is found to be induced by gravity, which has been defined as **gravikinesis**. However, unfortunately, this term does not comply with the accepted definition of the term kinesis, since it is not independent of the direction of the stimulus (in this case, the direction of the gravitational field). In a flat, horizontal cuvette, microorganisms are forced to swim horizontally. Without any other vectorial stimulation, the cells will move at the same velocity V_1 (with some statistical deviation), irrespective of their direction (Fig. 1.2a). In a vertical cuvette, the cells will simultaneously swim and sediment, since they usually have a higher intracellular density than the surrounding medium (Fig. 1.2b). The force F that acts on the cells is

$$F = \frac{4}{3}\pi a^3 \Delta\rho g, \tag{1.1}$$

where a = radius of the object (assumed to be spherical), $\Delta\rho$ = the difference in specific density between object and surrounding medium, and g = gravitational acceleration. The resulting sedimentation velocity is (Stokes law)

$$V_s = \frac{2a^2\Delta\rho}{9e}. \tag{1.2}$$

In upward swimming cells, the sedimentation velocity V_s will vectorially subtract from the locomotion speed V_1, and in downward swimming cells, add to the individual swimming speed. Horizontally swimming cells should not be affected, except for a downward sedimentation that would slightly bend the horizontal

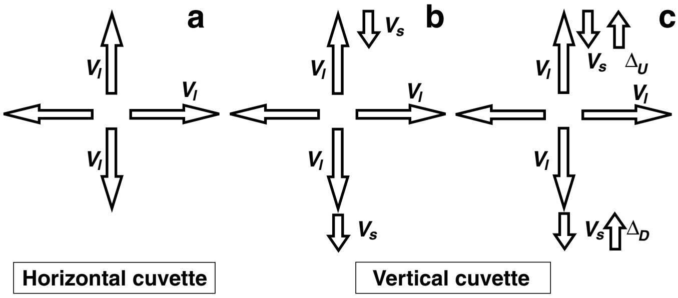

Figure 1.2. Diagram to explain the observed gravikinesis in certain ciliates. In a horizontal cuvette, all cells swim with about the same velocity (**a**). In cells without gravikinesis, the sedimentation velocity adds vectorially to the upward or downward swimming velocity (**b**). In contrast, in cells with gravikinesis, the effect of sedimentation is partially, completely, or overcompensated by an increased upward swimming velocity and a lowered downward swimming velocity (**c**).

path downward (Nagel & Machemer, 2000b). This predicted behavior is found in the flagellate *Euglena* (Häder, 1996b; Vogel et al., 1993).

In certain ciliates – such as *Paramecium, Didinium, Tetrahymena*, and *Loxodes* – computer-aided image analysis and statistical evaluation of a large number of cells indicated a different result (Hemmersbach-Krause et al., 1993b; Machemer et al., 1991; Ooya et al., 1992). In upward swimming cells, sedimentation is compensated by an increased swimming velocity (kinesis); and, in downward swimming cells, a slower swimming velocity is observed (Fig. 1.2c; Machemer et al., 1991). The downward velocity V_D modified by the gravikinetic component Δ_D is

$$V_D = V_1 + V_S - \Delta_D \tag{1.3}$$

and the upward velocity V_U modified by the gravikinetic component Δ_U is

$$V_U = V_1 - V_S + \Delta_U, \tag{1.4}$$

where V_S = sedimentation velocity; Δ_U, Δ_D = gravikinetic components during upward and downward swimming, respectively; and V_1 = swimming velocity independent of the gravitational influence. The gravikinetic component Δ is

$$\Delta = (V_D - V_U)/2 - V_S. \tag{1.5}$$

Compensation of sedimentation can be either complete ($V_S = \Delta$; e.g., *Loxodes*), partial ($V_S > \Delta$; e.g., *Paramecium* and *Didinium*), or even overcompensated ($V_s < \Delta$; e.g., *Tetrahymena*). The same species may even have different compensation rates, depending on the age of the culture (Bräucker et al., 1994). The gravikinetic effect, however, is independent of the swimming velocity, because it is the same in slow and fast swimmers (Machemer & Machemer-Röhnisch, 1996).

It has been speculated that gravikinesis is due to the pressure of the cytoplasm onto the respective lower membrane, which activates mechanosensitive channels. In the rear end, there are hyperpolarizing channels in the membrane and in the front end depolarizing ones. Hyperpolarization of the cell results in an increased

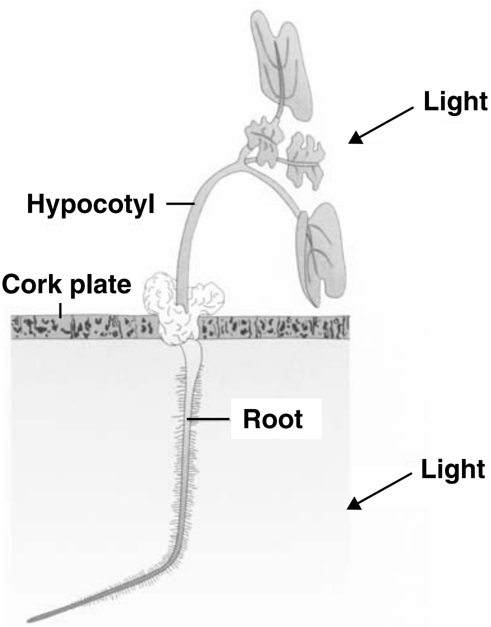

Figure 1.3. Positive and negative phototropism in the shoot and root, respectively, of a mustard seedling (*Sinapis alba*). The leaves are oriented perpendicular to the impinging light beam (diaphototropism) (modified from Nultsch, 2001).

swimming velocity and depolarization in a decreased one (Baba et al., 1991; Machemer et al., 1991; cf. Chapters 4 and 9). Fraenkel and Gunn (1961; Gunn et al., 1934) made a distinction between orthokinesis (which they defined as a change in linear velocity) and klinokinesis (which is an alteration in the frequency of directional changes or angular velocity). These definitions have been used by some zoologists.

1.2.2 Behavioral responses of sessile plants to environmental stimuli

Higher and lower sessile plants also show movement responses to environmental stimuli. Like phototaxis in motile microorganisms, phototropism is a steady-state bending of an organ of a sessile plant with respect to the direction of the light source. Shoots of higher plants bend toward the light source (positive phototropism), while roots either bend away from it (negative phototropism) or are indifferent toward light (Fig. 1.3). Lateral branches and leaves usually orient themselves perpendicular or at a different angle with respect to the impinging light beam (dia- or transversal phototropism). In some plants, leaves, flowers, or shoots follow the course of the sun over the day, which is called sun tracking or heliotropism (Koller, 2001). Also, organs in lower plants bend with respect to the light direction [e.g., the sporangiophores (vertical aerial hyphae that carry the sporangium) in the mold *Phycomyces* (Galland, 2001)].

Cambridge University Press
0521820529 - Gravity and the Behavior of Unicellular Organisms
Donat-Peter Hader, Ruth Hemmersbach and Michael Lebert
Excerpt
More information

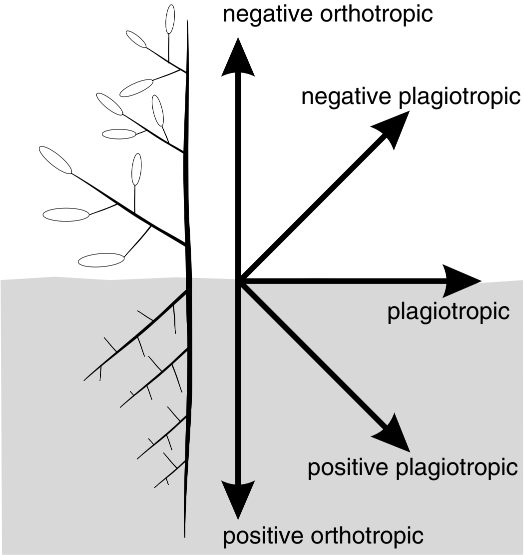

Figure 1.4. Positive and negative gravitropism of primary roots and shoots, respectively, of higher plants. Secondary roots and branches orient themselves at an angle (plagiotropic) to the gravitational force, and tertiary roots and shoots, as well as leaves, are often agravitropic (modified after Lüttge et al., 1994).

Without light stimulation, organs of higher plants respond to gravitactic stimulation. Primary shoots show negative (orthotropic) **gravitropism** and grow upward, primary roots positive gravitropism and consequently grow downward. Lateral branches and roots – as well as leaves – some flowers and fruits orient more or less perpendicular (plagiotropic) to the gravitational field (Fig. 1.4). The so-called setpoint angle (Edelmann et al., 2002) is controlled by two antagonistic forces – negative gravitropism and **epinasty** – which is an endogenous force in the direction opposite to gravitactic bending (Kang, 1979). This can be demonstrated by rotating a plant such as a *Coleus* slowly with its shoot in horizontal orientation (cf. clinostat principle, Chapter 2). By this means, the gravitational stimulus is randomized, and the epinasty causes the leaves to bend downward.

As in motile microorganism, tropisms to other stimuli are described by a prefix such as

- mechano- (response to mechanical forces)
- gravi- (response to gravity)
- thigmo- or hapto- (response to touching an object)
- rheo- (response to streaming water or air)
- seismo- (response to vibrations)
- chemo- (response to a chemical stimulus)
- galvano- (response to an electrical stimulus)

Nondirectional responses to environmental stimuli are called **nasties**. A sudden decrease in solar radiation by a passing cloud can cause some flowers to close (photonasty); a seismic stimulus of a pollinating insect may induce the stamina

Cambridge University Press
0521820529 - Gravity and the Behavior of Unicellular Organisms
Donat-Peter Hader, Ruth Hemmersbach and Michael Lebert
Excerpt
More information

to bend inward and deposit their pollen onto the animal. Like phobic responses, nastic reactions are independent of the stimulus direction. A *Mimosa* leaf closes always in the same direction independent of the direction of the mechanical, seismic, chemical, or thermal stimulus.

1.2.3 "Microgravity" and hypergravity

Although an internationally accepted value for normal acceleration due to gravity on Earth exists (9.80665 m s^{-2}), there is no agreed-upon symbol when it is used as a unit. One can find (*g*), which can be confused with gram or (G) that might lead to confusion with the gravitational constant. We adopted the conventional usage "× *g*." To describe a reduced acceleration condition ($<1 \times g$) – e.g., in a spacecraft – different terms are being used and discussed. Besides weightlessness or $0 \times g$, assuming that the residual acceleration is not perceived by the system, the terms **low *g***, **hypogravity**, and **microgravity** are used. Microgravity does not correspond to $10^{-6} \times g$, but indicates low gravity (Hammacher et al., 1987) and takes the residual acceleration due to, e.g., movement of the astronauts, into account.

1.3 Ecological significance

It is an obvious advantage for a higher plant to orient in the Earth's gravitational field so that the shoot grows upward to reach the top of the canopy to harvest solar energy for its photosynthetic apparatus. It is also advantageous for the roots to grow downward into the soil to reach the water table. Likewise, photosynthetic motile microorganisms have an advantage when they swim upward in the water column to reach the surface to have access to sunlight. Also, nonphotosynthetic microorganisms gain from gravitactic orientation (e.g., to find their mating partners at the surface) to find food and oxygen at a certain layer in the water or to find the bottom to settle and become sessile or to find microaerophilic conditions. However, this is a limited view of a much more complex ecological situation.

As we will see in the upcoming chapters, microorganisms respond to a multitude of external stimuli, including light and gravity, temperature and chemical gradients, the magnetic field of the earth, fluid currents, and even electric fields (Berman & Rodhe, 1971; Kamykowski & Zentara, 1977). The final response of the organisms to these multiple stimuli may be a vectorial addition, or one stimulus overrides the response to the others or the answer is the result of a complex network.

As an example, in darkness, photosynthetic flagellates swim upward, guided by the gravitational field of the Earth. This behavior is of ecological advantage, because it will bring the cells to the top of the water column to be near the surface when the sun rises. The upward orientation is supported by positive phototaxis (movement toward a light source), as soon as light at low irradiances is available. In contrast, at high solar radiation, negative phototaxis takes over in many organisms, which leads the cells away from the surface. This is a reasonable