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Bacterial self-organization: co-enhancement of complexification and adaptability in a dynamic environment

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During colonial development, bacteria generate a wealth of patterns, some of which are reminiscent of those occurring in abiotic systems. They can exhibit rich behaviour, reflecting informative communication capabilities that include exchange of genetic materials and the fact that the colony's building blocks are biotic. Each has internal degrees of freedom, informatic capabilities and freedom to respond by altering itself and others via emission of signals in a self-regulated manner.

To unravel the special secrets of bacterial self-organization, we conducted an integrative (experimental and theoretical) study of abiotic and biotic systems. Guided by the notion of general biotic motives and principles, I propose that the informative communication between individuals makes possible the enhancement of the individuals' regulated freedom, while increasing their cooperation. This process is accomplished via cooperative complexification of the colony through self-organization of hierarchical spatio-temporal patterning. The colonial higher complexity provides the degree of plasticity and flexibility required for better colonial adaptability and endurability in a dynamic environment. The biotic system can modify the environment and obtain environmental information for further self-improvement. I reflect on the potential applications of the new understanding on 'engineered self-organization of systems too complex to design' and other issues.

Keywords: information; communication; regulated freedom; complexification; flexibility-based adaptability; dynamic environment

1. Prologue

Bacteria have come up with sophisticated modes of cooperative behaviour to cope with adverse and varying environmental conditions. They developed intricate communication capabilities, including a broad repertoire of chemical signalling mechanisms, collective activation and deactivation of genes and even exchange of genetic materials. With these tools they can communicate and self-organize their colonies into multicellular hierarchical aggregates, out of which new abilities emerge (Nester *et al*. 2001; Caporale 1999; Rosenberg 1999; Joset & Guespin-Michel 1993; Shapiro & Dworkin 1997; Shapiro 1988, 1995; Albrecht-Buehler 1990; Losick & Kaiser 1993, 1997; Ben-Jacob 1997; Ben-Jacob *et al*. 2000*a*; Bassler 2002).

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One such emergent ability is the multiple drug resistance that has become a major health problem worldwide. Resistance can arise through cooperative response using sophisticated communication capabilities: bacteria in self-organized colonies can be far more resistant to antibiotics than the same bacteria in suspension. In the colony, they form a genomic web as they transfer the resistance information by means of plasmids or viruses within and between colonies, and even to foreign bacteria (Dixon 1994; Baron 1996; Shapiro 1992; Miller 1998; Levy 1998; Ben-Jacob *et al.* 2002).

There are potential applications of studying bacterial self-organization. It might help develop novel fighting strategies, such as drugs that impair bacterial communication capabilities (Ben-Jacob *et al.* 2002). On the other hand, means to enhance bacterial cooperation may improve useful industrial processes, such as the decomposition of waste products and drug production. There are also purely intellectual rewards to unravelling the secrets of bacterial self-organization. Clues and percepts elicited from bacterial studies might shed some light on the puzzles of natural self-organization and complexification, and even on the emergence of cognition (Bloom 2000; Kauffman 1995, 2000; Penrose 1994, 2002; Deblrück 1986).

Diverse open systems, biotic and abiotic alike, respond to externally imposed conditions by forming complex hierarchical spatio-temporal patterns (Thompson 1944; Stevens 1974; Ben-Jacob & Garik 1990; Ben-Jacob 1993; Ben-Jacob & Levine 1998, 2001; Ball 1999; Schwetzer 1997). In the early 1950s, Alan Turing understood that complex structures emerge in open systems only when there is competition between two or more tendencies (Turing 1952). He thus started a new field—self-organization—and set its first principle, of patterning via competition.

Often, the competition is regarded as being between the approaches towards global equilibrium (global entropy production) and local equilibrium (local entropy production). The global kinetics drives the system towards decorated, irregular, scale-invariant shapes, while the local dynamics imposes local characteristic length-scales and overall symmetries (Ben-Jacob & Garik 1990; Ben-Jacob 1993; Ben-Jacob & Levine 2001).

When the system is driven farther from equilibrium, the global tendency is intensified but, at the same time, it also amplifies the local effects, which then act as a singular perturbation capable of affecting the macro-level organization. By the same token, the global tendency, acting as singular feedback, can reach down and affect the micro-level organization by favouring one particular small-scale structure over the others. Thus, the balance in the competition is determined via singular interplay between the two levels (Ben-Jacob & Garik 1990; Ben-Jacob 1993; Ben-Jacob & Levine 2001; Kessler *et al.* 1998; Langer 1969). The two-level picture is often insufficient. In such cases, a hierarchical multi-level organization is the only possible answer to the requirement of self-consistency (solvability).

During colonial development, bacteria create a wealth of patterns, some of which are reminiscent of those observed in abiotic systems. Therefore, it could be expected that the principles of abiotic self-organization would help us to understand the colonial behaviour. Still, some words of caution are warranted here. One must avoid 'physification' or 'mathematification' of biotic systems, and keep in mind that bacteria can exhibit much richer behaviour that reflects their sophisticated communication capabilities.

As in abiotic systems, patterns emerge through the singular interplay between individual bacteria (micro-level) and the colony (macro-level). However, inherent in

the growth of a colony is an additional degree of complexity: the building blocks are themselves biotic systems, each with internal degrees of freedom, internally stored information and internal processing ('interpretation') of information. These afford each bacterium a certain freedom to respond and alter itself, sometimes even by altering gene expression and bringing about changes in other bacteria via emission of signals in a self-regulated manner (Jones 1993; Stent 1975; Fuqua *et al.* 1994; Keller 1983; Ben-Jacob 1998).

The notions of freedom and cooperation are usually perceived as contradictory (Poundstone 1992). Bacteria solved this apparent paradox by forming complex hierarchical colonial structures. Such organization simultaneously elevates the degree of freedom of the individual cells and the level of cooperation. The 'smart' bacteria (Shapiro 1992; Ben-Jacob *et al.* 1997a) have 'realized' (over evolution) that increasing informative communication between individuals results in increased freedom *and* cooperation of the individuals. As the individuals increase their adaptability to the group, the colony elevates its adaptability and endurability by increasing its complexity (Ben-Jacob 1998). The essential new lesson that has been learned from bacteria is that colonial higher complexity provides the degree of plasticity and flexibility required for better adaptability and endurability of the colony, as a whole, to a dynamic environment (Huberman & Hugg 1986).

2. The principles of abiotic self-organization

In the early 1600s, Johannes Kepler marvelled at the graceful forms of snowflakes (Kepler 1916), perhaps the most striking example of interfacial pattern formation (or invasive self-organization) in abiotic systems. The origin of their sixfold beauty eluded him, but he did realize that it was due to some power inherent in matter, which he dubbed 'facultas formatrix'. The spontaneous emergence of patterns remained a mystery for researchers in the natural sciences long thereafter. The mist began to lift only after Alan Turing proposed his principle of patterning via competition. In snowflakes, the competition is between the diffusion of water molecules towards the flake and the microscopic dynamics at the interface.

The history of invasive pattern formation has been interesting and educative ever since (Ben-Jacob & Garik 1990; Ben-Jacob 1993). The next step in understanding snowflakes was the realization that microscopic dynamics act as a singular perturbation amplified by the diffusion process subject to the microscopic solvability criteria. It became clear then that the sixfold symmetry of the snowflake emanates from the sixfold symmetry of the underlying ice lattice (figure 1).

The macro-to-micro singular competition, the new self-consistency principle and the resulting hierarchy of integrated levels are even more evident during electrochemical deposition. The different morphologies on the macroscopic scale go hand in hand with the different micro-level organizations shown in figure 2.

An additional phenomenon exhibited in figures 3 and 4 is that of 'morphology transitions'. The balance between the global and local tendencies of the competition varies as the growth conditions change. The observed patterns may be grouped into a small number of typical or 'essential' shapes (morphologies), each representing a different dominant effect (figure 1). For a given system, each morphology is observed over a range of growth conditions. This brings to mind the idea of the morphology diagram, in analogy with the phase diagram for systems in equilibrium. Now, if the
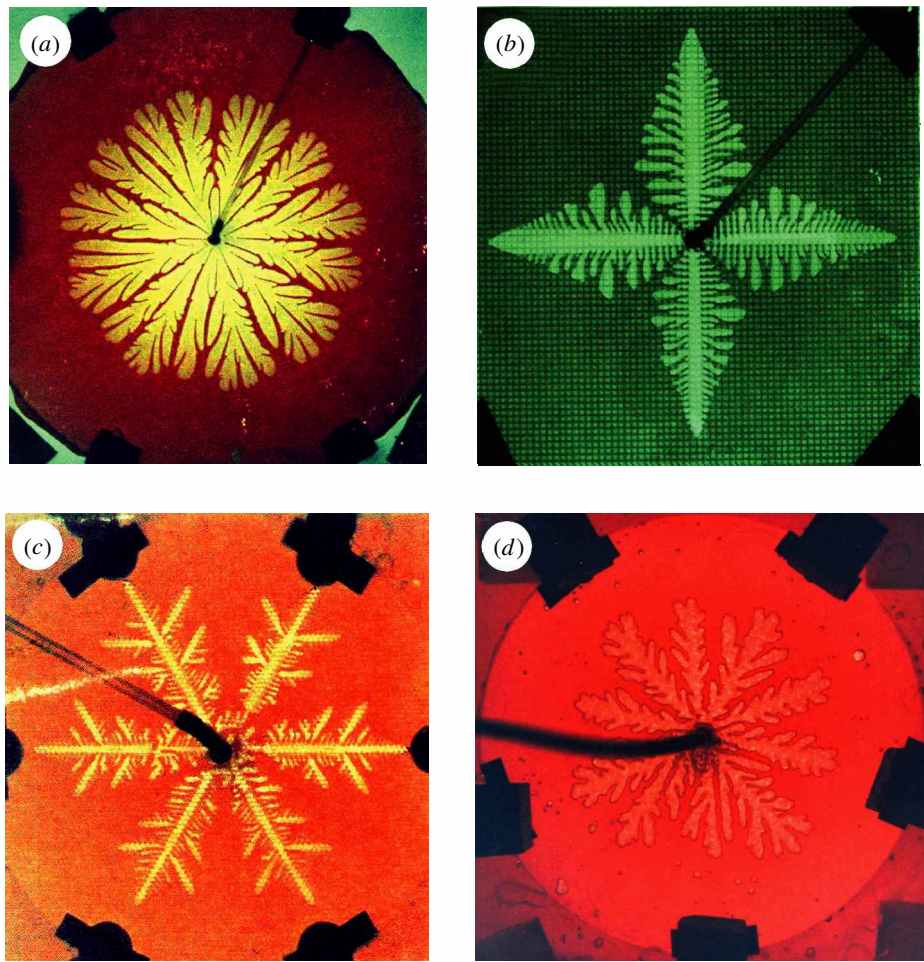
Figure 1. Demonstration of the microscopic solvability criteria and the idea of 'essential shapes' or morphologies via observations in a Hele-Shaw cell. The Hele-Shaw cell consists of two closely spaced Plexiglas plates sandwiching a layer of dyed glycerol. Through an inlet at the centre of the top plate, air is injected into the glycerol. Tip-splitting morphology is shown in (*a*). This is the typical morphology for isotropic conditions. The patterns in (*b*)–(*d*) resulted from anisotropy imposed on the growth by grooving a weak modulation on the bottom plate: a square lattice (fourfold symmetry), a triangular lattice (sixfold symmetry), and a quasi-periodic lattice (10-fold symmetry) are shown in (*b*), (*c*) and (*d*), respectively. Although weak, the imposed symmetries cause the air bubble to assume the dendritic morphology of 'airflakes' resembling: (*b*) solid CO_2 or fourfold 'Martian flakes'; (*c*) snowflakes; (*d*) quasi-periodic flakes. Note that the angles of the side-branches relative to the main trunk are (90°), (60°) and (36°, 72°) for (*b*), (*c*) and (*d*), respectively. This experiment provided important support to the idea of the existence of common principles in abiotic self-organization (Ben-Jacob & Garik 1990; Ben-Jacob 1993).

concept is to be meaningful, there should be a morphology-selection principle analogous to the minimum-free-energy selection principle in equilibrium. We proposed that, farther from equilibrium, the selected morphology is the fastest growing, since
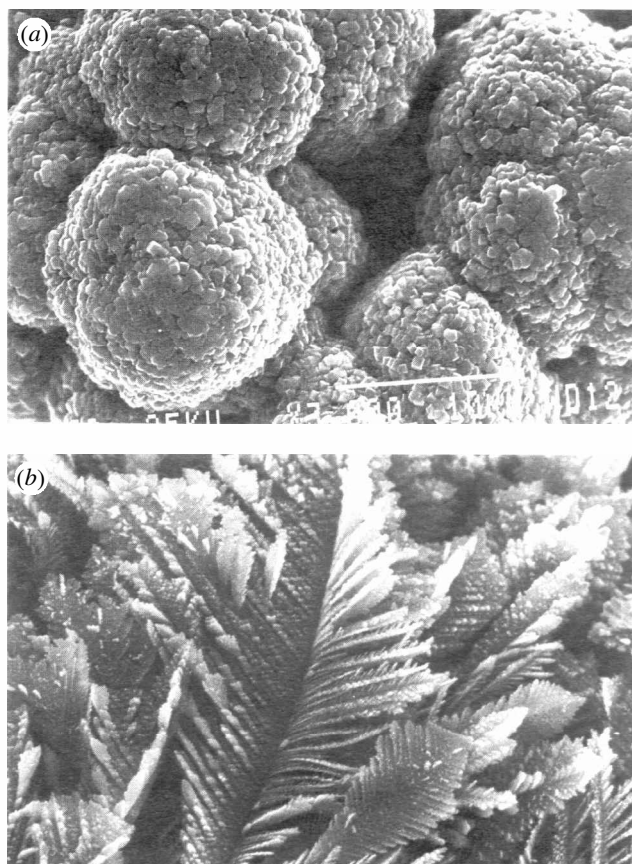
Figure 2. Demonstration of the macro-to-micro singular feedback in an electrochemical deposition (ECD) cell. Electron microscope view (magnification ×1000). (*a*) 'Bushy' branching morphology; (*b*) dendritic growth (see figure 3). Even at this magnification, several levels are observed. The 'bulbs' on top are aggregates of randomly oriented small crystals, and the dendrites shown at the bottom have side branches with side branches. On a lower scale, the dendritic growth has a different atomic lattice structure with different unit (Ben-Jacob & Garik 1990; Ben-Jacob 1993).

the global tendency dominates the growth. Closer to equilibrium, the selection is of simple, local, geometrical organization, since the local tendency is dominant (Ben-Jacob & Garik 1990; Ben-Jacob 1993). Such regular ordered patterns have relatively low complexity. Similarly, the disordered patterns very far from equilibrium are of relatively low complexity (Ben-Jacob & Levine 2001).

The ordinary notion of stability, as used in equilibrium, can also be applied to the case of open systems exhibiting static or regular steady states. It is not valid for morphologies with hierarchical spatio-temporal complex patterns formed during self-organization. In such cases, the morphology tolerance and system robustness are the relevant concepts rather than stability (Carlson & Doyle 1999; Zhou & Carlson 2000). Tolerance and robustness require higher plasticity and flexibility that go hand in hand with higher complexity.
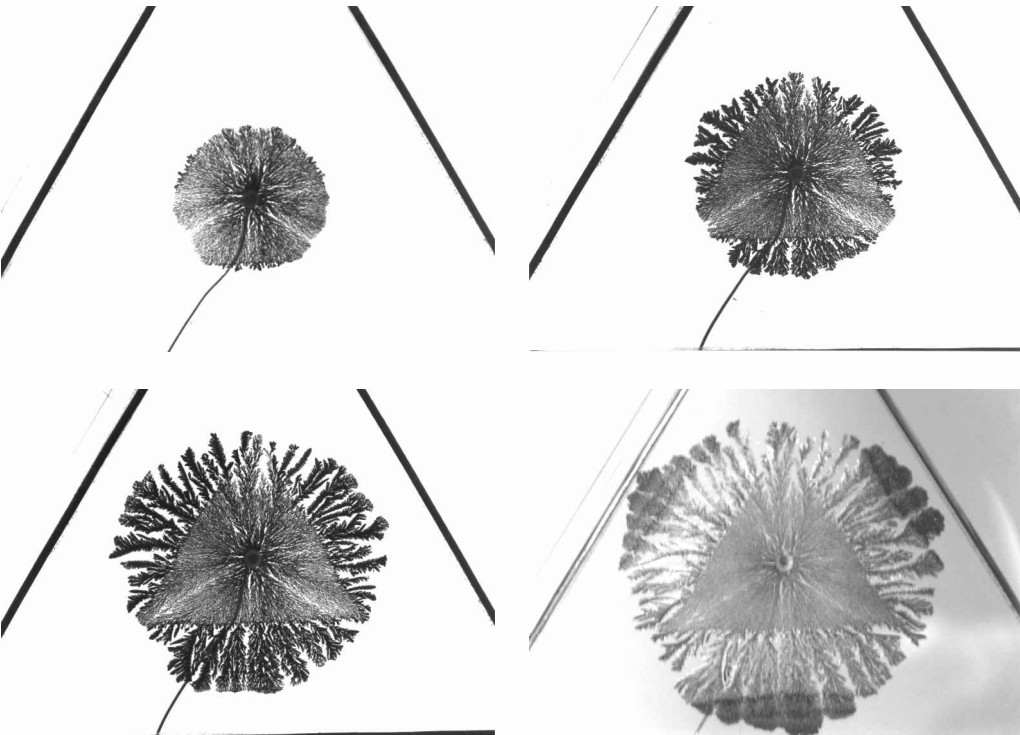
Figure 3. Morphology transitions between branching and dendritic growth in a triangular ECD cell. Although the branching growth has a circular envelope, the morphology transition between branching and dendritic growth (which occurs at a specific value of the electric field) forms sharp triangular boundaries between the morphologies. A similarly sharp transition is observed in the second transition back to dense branching growth. These observations also demonstrate that the growth pattern is less complex for both high and low values of the electric field. The electrodes are made of copper and the electrolyte is $ZnSO_4$. The back transition is due to the deposition of copper ions together with the zinc ions.

3. Observed repertoire of bacterial self-organization phenomena

(a) *Self-organization of lubricating bacteria*

Lubricating bacteria can move on hard surfaces by cooperative production of wetting fluid in which they swim. Under imposed growth conditions of starvation and hard surfaces, these bacteria face a Turing competition between the global nutrient diffusion and the local bacterial organization for the sake of lubrication and movement. They solve the competition by generating branching colonial organization (Ben-Jacob 1997; Ben-Jacob *et al.* 2000*a*, *b*). Examples of three branching morphotypes are shown in figures 5–7.

(i) *Simple branching*

As the peptone (food) level is lowered, the colonial patterns shift from compact to tip splitting, reminiscent of the viscous fingering observed in the Hele-Show system,
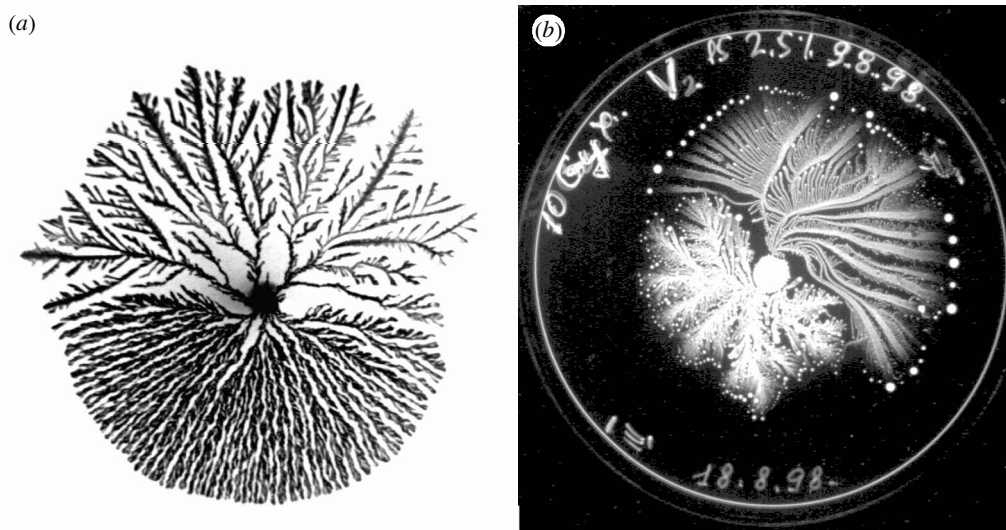
Figure 4. Demonstration of flexibility, tolerance and robustness of the morphologies (*a*) in ECD and (*b*) in growth of the vortex morphotype bacteria. In both cases, the growth conditions correspond to the boundaries between two morphologies. Accidental local asymmetry at the initial stage caused the initial growth of the two different morphologies. Then, each kept its characteristic velocity–complexity correspondence. The more complex morphology (under the given growth condition) is also the faster expanding.

to 'bushy' fractal patterns (figure 5). At even lower peptone levels, the colonies revert to more organized structures of fine radial branches.

Under the microscope, the bacteria at the outskirts of the colony appear active and perform random-walk-like swimming, while further inside they are stationary (pre-spore state), and even deeper inside they sporulate. Branching patterns were observed in other strains of lubricating bacteria: in *Serratia marcescens* and *Salmonella anatum* (Matsuyama & Matsushita 1991; Matsuyama *et al.* 1992) and in *Bacillus subtilis* (Fujikawa & Matsushita 1991; Matsuyama *et al.* 1993; Matsushita & Fujikawa 1990; Mendelson & Salni 1996).

(ii) *Chiral branching*

Bacteria display various chiral properties, including the flagella propeller-like movement resulting from the asymmetry of the molecular motors. Still, the observation of a pattern of strongly twisted, chiral branches, all with the same handedness, was unexpected (figure 6). First, it was generated by swimming bacteria. Second, it was generated by the same bacterial strain, *P. dendritiformis*, which also develops the simple branching morphotype. The morphotype transitions between simple and chiral branching are discussed in § 3. In general, transitions will occur into the morphotype whose colonies can expand faster and organize in a more complex pattern at the given growth conditions.

Optical microscope observations indicate that, in the case of chiral branching, the cells are much longer (figure 6) and the random walk turns into a quasi-one-dimensional random walk. The bacteria at the branch-tips exhibit minute twists, all with the same handedness.
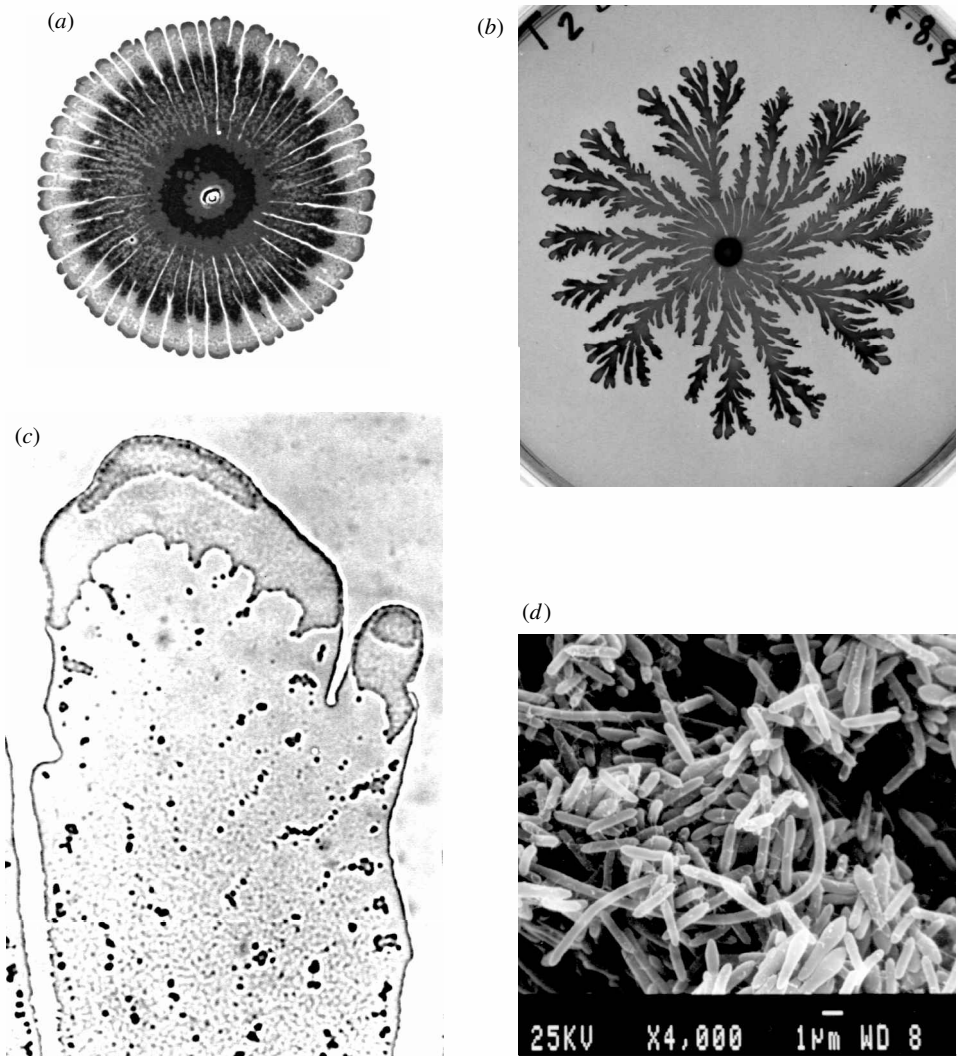
Figure 5. Patterns exhibited by the branching morphotype of the *Paenibacillus dendritiformis* bacteria: (*a*) tip-splitting growth at high peptone levels; (*b*) 'bushy' branching at intermediate levels; (*c*) a closer look at the patterns of density variations within tip-splitting branches. The latter manifests the additional level of organization between the individual bacteria and the branches. (*d*) An even closer look, through an electron microscope, reveals the large variations between cells due to their 'regulated freedom'. This picture was taken during a morphotype transition from branching to chiral. Note the appearance of very long bacteria.

We demonstrated (Ben-Jacob *et al.* 1995*a*, 2000*b*) that the flagella handedness, while orientational interaction is taking place between long bacteria, acts as a singular perturbation that generates the colonial chiral organization. It does so in a manner similar to that in which ice crystalline anisotropy imposes the observed six-fold symmetry of snowflakes. The added element is that the colony can affect the gene expression of its own bacteria, to elongate them and thus activate the singular perturbation required for chiral self-organization.
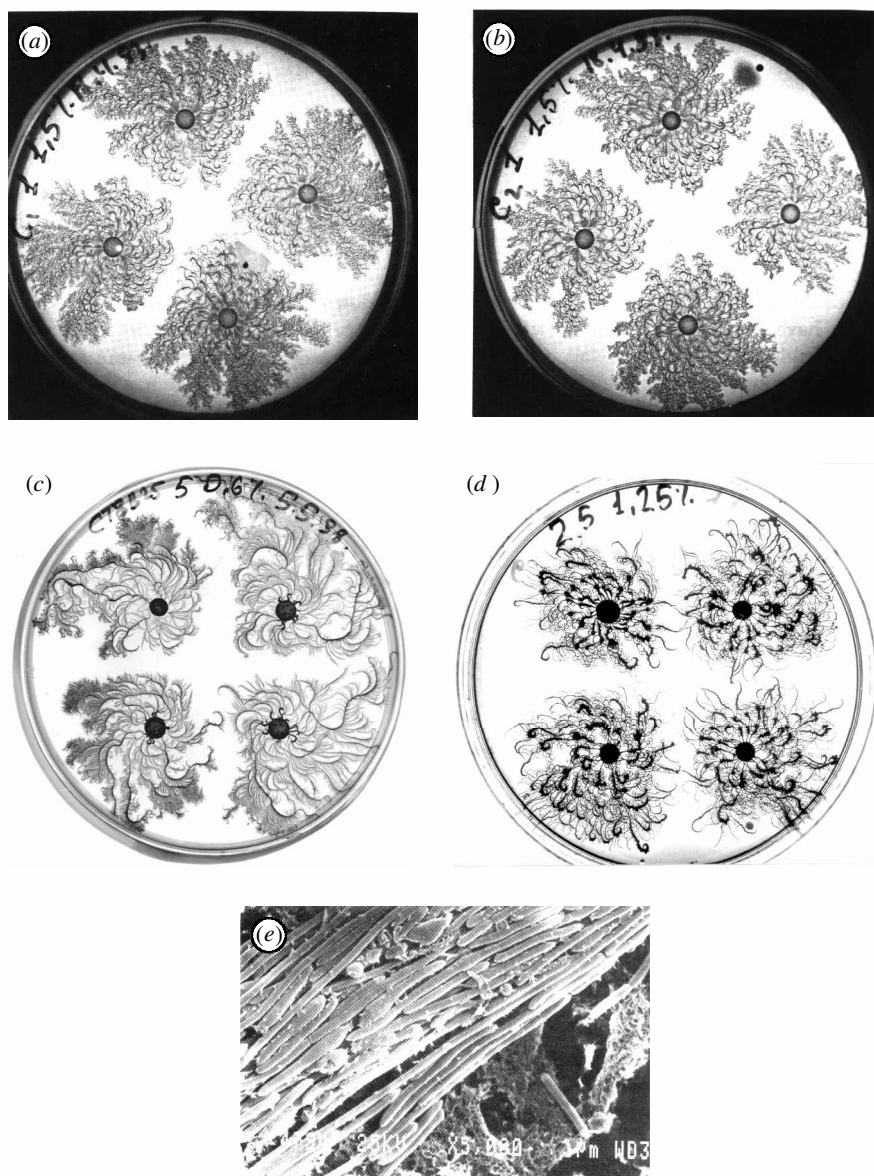
Figure 6. Patterns exhibited by the chiral morphotype of the *P. dendritiformis* bacteria. Different growth conditions lead to the formation of different patterns. The similarity of the four colonies on each plate demonstrates their tolerance and robustness and indicates that their high complexity is not accidental. Even more impressive is the similarity between the two plates (*a*) and (*b*), demonstrating the reproducibility of our experiments of growth at the same levels of peptone and agar. The growth conditions are (*a*) and (*b*) $1 \, \mathrm{g} \, \mathrm{l}^{-1}$ peptone and 1.5% agar, (*c*) $5 \, \mathrm{g} \, \mathrm{l}^{-1}$ peptone and 0.6% agar and (*d*) $2.5 \, \mathrm{g} \, \mathrm{l}^{-1}$ peptone and 1.25% agar. Comparison of the plates demonstrates the differences in the colony's organizational complexity. The reduced growth towards the centre in plates (*a*) and (*b*) is because of the mutual effect of repulsive chemotactic signalling. The more complex-looking colonies (*d*) are less affected. (*e*) Electron microscope view of the orientational co-alignment of these long bacteria.
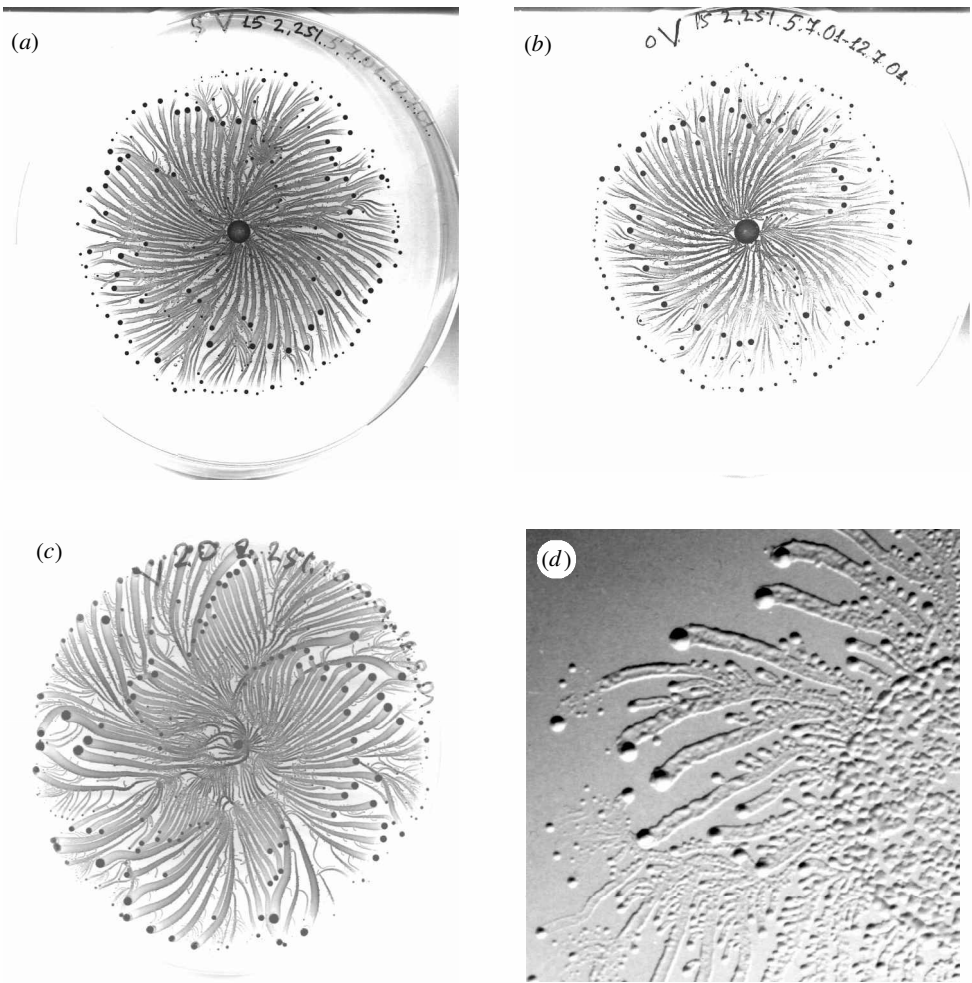
Figure 7. Observations of the *P. vortex* bacteria. Plates (*a*) and (*b*) were grown at the same peptone level (15 g l^{-1}) and agar concentration (2.25%). It is an additional demonstration of the flexibility and robustness of these complex morphologies. The two plates appear very similar. However, closer inspection reveals that (*a*) is slightly more ordered. Such variations originate from small changes in the solution in which the bacteria were grown before inoculation on the petri dishes. It demonstrates the colonial memory (discussed in § 5). The additional patterns in (*c*) and (*d*) show the effect of growth conditions.

(iii) *Vortex branching*

More than 50 years ago, observations of collective migration of *B. circulans* on hard surfaces showed turbulent-like bacterial flow with eddy (vortex) formation, merging and splitting of vortices, rotating 'bagels', and more (Smith & Clark 1938; Wolf 1968). All of these phenomena are also observed in the self-organization of our new *P. vortex* strain when growing on a very hard surface. The self-generation of vortices provides high bacterial density to facilitate movement on such surfaces (video available from the author upon request; Ben-Jacob *et al.* 1997*b*, 1998). Vortex size can range from tens to millions of cells circling in coordination around a common

centre, at a cell speed of *ca.* $10\,\mu\mathrm{m\,s^{-1}}$. Both clockwise- and anticlockwise-rotating vortices are observed within the same colony. Once formed, the vortex expands and translocates as a unit, leaving behind a trail of cells and forming its own new branch.

(iv) *Morphology diagram and morphology selection principle*

Each of the morphotypes exhibits a wealth of different patterns for different peptone levels and agar concentrations. These observations may be organized in a morphology diagram, with pattern-velocity (rate of colonial expansion) correspondence and velocity discontinuity upon transition between morphologies. The implication is that the selection of the most flexible morphology should occur also during bacterial self-organization (Ben-Jacob 1997; Ben-Jacob *et al.* 2000*a*).

(*b*) *Bacterial self-organization in suspension*

(i) *Self-organization under oxidative stress*

Escherichia coli is perhaps the most studied bacterial strain (Nester *et al.* 2001; Caporale 1999; Rosenberg 1999; Joset & Guespin-Michel 1993), and yet the cooperative behaviour of these bacteria continues to generate surprises. *E. coli* can be stimulated to develop patterns of spots, stripes and rings in colonies grown under oxidative stress that triggers colonial cooperative behaviour (Budrene & Berg 1991, 1995). Similar patterns are also developed during colonial growth of *S. typhimurium* under similar growth conditions (Blat & Eisenbach 1995). The formation of spots, stripes and rings of high bacterial density helps the bacteria cooperate in decomposing waste products and thus relieve the oxidative pressure.

(ii) *Bacterial convection patterns*

In the case of high density of aerobic bacteria in fluid, the stress is insufficient oxygen. To solve this problem, the bacteria develop collective behaviour that produces convection in the fluid. This 'bioconvection' allows each cell to be exposed periodically to regions of the fluid that are rich in oxygen (Kessler & Wojciechowski 1997; Kessler & Hill 1997).

(*c*) *Spatio-temporal colonial patterning of gene expression*

Intricate patterns of gene expression were observed even in *E. coli* colonies without apparent geometrical structure (Albrecht-Buehler 1990). In more recent studies of the morphology diagram and patterns of gene expression in colonies of *B. subtilis*, it was discovered that, under certain growth conditions, the patterns of gene expression diverge significantly from the geometrical pattern (Mendelson & Salni 1996). Apparently, in some cases, several rings of bacteria activate a gene, and in others, the gene-expression activity propagates back and forth along branches of stationary bacteria. These observations provide a direct demonstration of colonial genetic communication.

Under ordinary growth conditions, colonies of *Proteus mirabilis* develop a very stable terrace structure of concentric rings. It turns out that the bacterial cells switch identities from swarmers to swimmers and back. At a certain cue, as yet unknown,

the swimmer cells stop dividing and grow into elongated, hyper-flagellated swarmers. The swarmers form collective 'rafts' (reminiscent of *P. vortex*) that can move efficiently on the surface. After a while, these cells differentiate back to swimmers, filling in new regions of a 'consolidation phase' (Shapiro 1988, 1995; Albrecht-Buehler 1990; Rauprich *et al.* 1996). These dynamics form very smooth rings compared, for example, with the ring structures observed in *B. subtilis*, which are quite ragged. This implies colonial regulation capable of generating synchronized gene expression.

The predator myxobacteria afford the richest set of phenomena observed during colonial development, including cooperative feeding on other bacteria, group motility, cell differentiation, aggregation and cohesion, rippling and formation of fruiting bodies for a more efficient dissemination of spores in response to starvation (Shapiro & Dworkin 1997; Albrecht-Buehler 1990; Losick & Kaiser 1997; Ben-Jacob *et al.* 2000*a*; Rosenberg 1984; Kuner & Kaiser 1982; Dworkin 1999). All of these phenomena bear amazing similarity to those exhibited by the eukaryote *Dictyostelium* amoebae, which is genetically much closer to us than to myxobacteria (Ben-Jacob *et al.* 2000*a*). This makes sense when we remember that both amoebae and myxobacteria live in a similar environment and feed on bacteria. The challenge is to understand how cell behaviour is coordinated in a self-consistent manner with gene expression, in order for multicellular behaviour to emerge.

4. Using models for further understanding

In physics and chemistry, models have long served as an indispensable research tool, while, in the life sciences, many still question the value and predictive power of models. It is true that simulations alone, without analysis and close comparison with observations, are insufficient; one may easily catch Cowan's 'reminiscence syndrome': the tendency to devise a set of rules that mimic some features of the observed phenomenon and mistake it for a proper model (Horgan 1995). At the other end there awaits the 'realistic trap', when the model becomes so swamped with detail and unknown parameters that it looses any predictive power. The way to avoid those pitfalls is the 'generic modelling' approach that requires understanding of the fundamental mechanisms at the heart of the phenomenon. The idea is to elicit, from the observations and the knowledge about general biotic motives and principles, the generic features to be included in the model. Then the model can really help in acquiring new understanding and making reliable new predictions for future tests (Ben-Jacob 1997, 2002; Ben-Jacob *et al.* 1994*a–c*, 2000*a,b*; Camazine *et al.* 2001; Kozlovsky *et al.* 1999; Adler 1969; Berg 1993; Lackile 1986).

(*a*) *Modelling chemotactic response*

Chemotaxis is cell movement in response to gradients in chemical concentration (Ben-Jacob *et al.* 1995*a,b*; Czirok *et al.* 1996; Cohen *et al.* 1996). The movement can be biased either towards (attractive) or away from (repulsive) higher concentration. Swimming bacteria implement chemotaxis by decreasing the tumbling frequency as they swim up the gradient of an attracting chemical or down the gradient of a repelling agent. Chemotaxis towards food is a well-studied example. The chemotactic response can also be due to chemicals produced directly or indirectly by the bacteria, a phenomenon we referred to as chemotactic signalling (Adler 1969; Berg 1993; Lackile 1986; Ben-Jacob *et al.* 1994*c*).
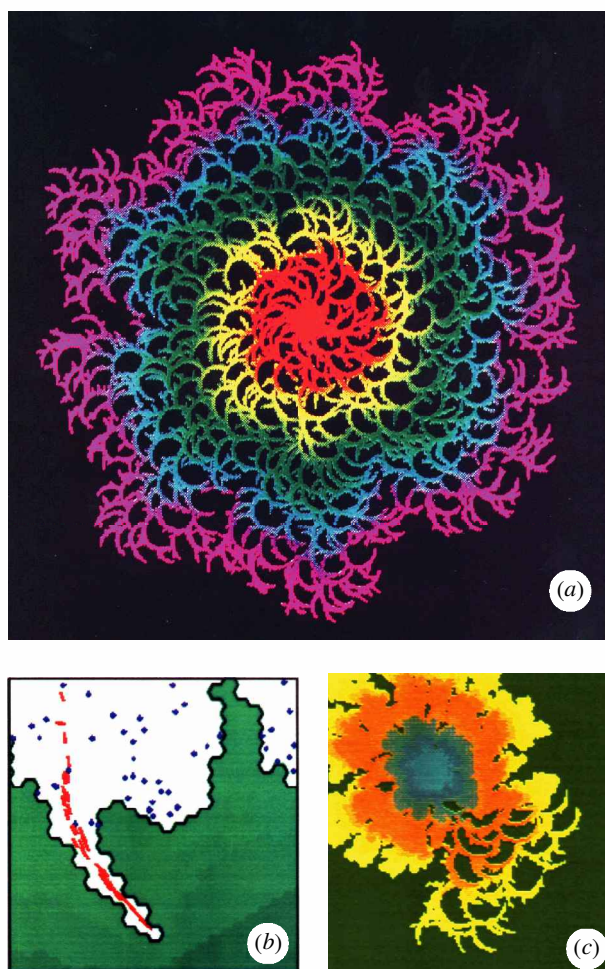
Figure 8. Results of numerical simulations of (*a*) the chiral patterning and (*c*) the morphotype transitions from branching to chiral patterns that are discussed in the text. The different colours in (*a*) are different time stages. The small blue squares in (*b*) are the branching morphotype bacteria and the red bars are the chiral morphotype bacteria.

With generic modelling, we were able to conclude that three kinds of chemotactic response were involved in the self-organization of *P. dendritiformis*: attractive, short-range chemotactic signalling; nutrient chemotaxis; and repulsive, long-range chemotactic signalling. We propose that

(i) the fine radial branching growth results from domination of the chemorepellent response;

(ii) the transition to the 'bushy' fractal branching growth corresponds to the turning on of the food chemotaxis above a threshold level;

(iii) the internal observed patterns of the tip-splitting branches are a chemoattractive response.

Figure 9. The effect of antibiotic on the self-organization of the vortex-branching morphotype. (*a*) Normal growth; (*b*) with antibiotic. The change in pattern reflects amplification of both the repulsive (fewer and straighter branches and faster expansion) and the attractive (larger vortices) chemotactic response. The larger vortices help reduce the exposure of each bacterium to the antibiotic. The faster expansion is an 'attempt to escape' the antibiotic, which under natural conditions is not homogeneously spread.

The latter leads also to stronger emission of a long-range repellent by bacteria farther from the front, because of the fast depletion of food due to bacteria aggregation near the front. We tested these ideas using both the continuous nonlinear diffusion model and the discrete communicating-walkers model and obtained the observed behaviour (Camazine *et al.* 2001; Cohen *et al.* 1996; Kozlovsky *et al.* 1999; Ben-Jacob *et al.* 1994*c*, 2000*a*; Adler 1969; Berg 1993; Lackile 1986).

(*b*) *From flagella handedness to chiral branching*

It is known that flagella have a specific handedness. This has no effect on the tumbling of short bacteria or in bulk but, for long bacteria swimming in a thin layer of lubrication fluid, the rotation is planar and has specific handedness. In addition, the orientational interaction (co-alignment) of long bacteria limits the rotation to less than 90°, thus turning the two-dimensional random walk (of short bacteria) into a quasi-one-dimensional movement back and forth as the bacteria elongate. The three effects together impose a very weak chiral asymmetry on the advancement of the branch-tips. We assume that this asymmetry acts as a singular perturbation in a manner similar to that of the crystalline sixfold symmetry of the snowflake, thus leading to the chiral branching. This is why chiral branching goes hand in hand with longer bacteria. Testing these ideas in a 'communicating spinors' model (an appropriate version of the communicating-walkers model) resulted in the formation of chiral patterning (Ben-Jacob *et al.* 1995*a*, 2000*a*, *b*), as shown in figure 8.

Bacterial self-organization under antibiotic stress. Studying the effect of antibiotic stress on colonial self-organization, we discovered some remarkable phenomena of the kind shown in figure 9. With the aid of modelling we discovered that, in order to cope with the additional stress, the bacteria intensify their cooperation by co-amplifying both the attractive and repulsive chemotactic signalling (Ben-Jacob *et al.* 2002).
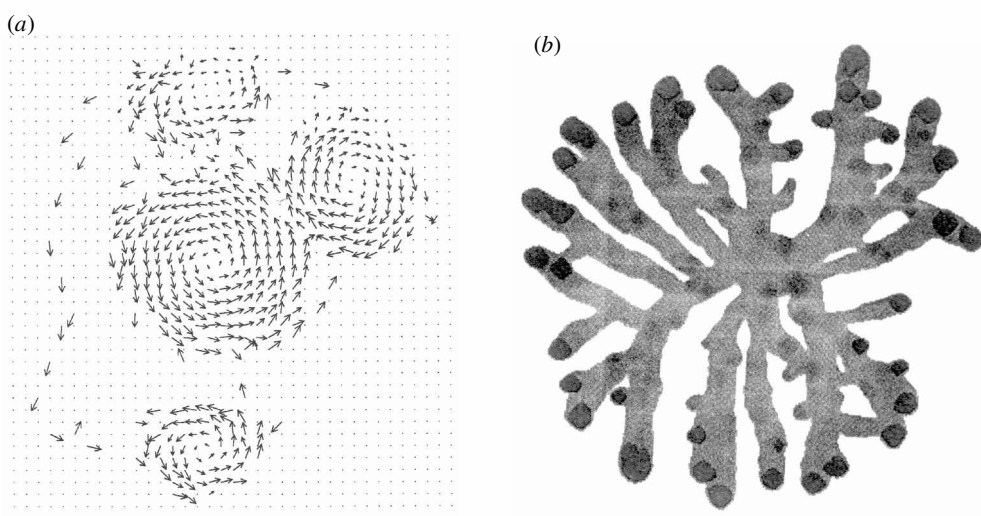
Figure 10. Results of numerical simulations of (*a*) the self-generation of vortices via attractive chemotactic signalling and (*b*) the organized colony in which the vortices are 'pushed out' by repulsive chemotactic signalling. The arrows indicate the magnitude and direction of the bacterial velocity. Both clockwise and anticlockwise vortices are generated, each forming a branch with a different twist.

Bacterial self-organization in the presence of antibiotics is a nice demonstration of our proposed principle of 'complexity → flexibility → adaptability'.

The complexity of the vortex colony provides the flexibility to alter its organization when an antibiotic is detected. The new organization has simpler structural complexity but higher operational complexity (concepts defined in §4). The altered organization enables the colony to expand faster and more efficiently in an attempt to escape the antibiotic. We also observed that colonies with lower complexity have a harder time coping with an antibiotic.

(*c*) *Demonstration of generality*

(i) *Self-generated chemotactic response*

Self-generated, short-range chemoattractant together with long-range chemo-repellent aid the self-organization of *P. dentritiformis*. This combined mechanism, together with additional motives, such as collective (autocatalytic or co-generation) generation of the chemical agents, secretion above a threshold level and response above a threshold level of concentration, appears to be general. Antibiotic stress is handled through a similar response. Another example is the vortex-branching organization of the *P. vortex* bacteria. We showed (Lackile 1986; Ben-Jacob *et al.* 2000*a*; Czirok *et al.* 1996) that a self-generated, short-range chemoattractant is used for the vortex formation, and a long-range chemorepellent pushes them outward, letting the colony expand (figure 10). The patterns emerging under oxidative pressure provide an additional example of the role played by a self-generated chemotactic response in bacterial self-organization (figure 11). The self-generated chemotaxis is used throughout the biological world, from bacteria through *Dictyostelium* amoebae to the self-organization of the human brain. Even the cAMP
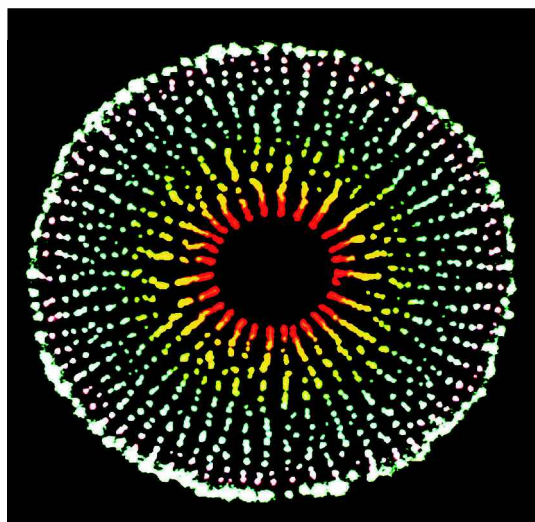
Figure 11. Results of numerical simulations of self-organization under oxidative pressure.
The radial organization in this example is a result of higher repulsive signalling.

used by amoebae is used also by the nervous system for neurite navigation,
with the ability to generate spiral waves of cAMP (Segev & Ben-Jacob 1998,
2000).

(ii) *Bursts of sectors in expanding colonies*

Bursts of mutant sectors during colonial development are a well-known phe-
nomenon. The sectors display different geometrical organizations. Combining exper-
imental observations and model simulations (figure 12), we were able to understand
the effect of various factors on the segregation of the mutant population, such as
the colonial geometry, self-generated chemotactic response, bacterial movement and
more (Golding *et al*. 1999).

The organizations of the bursting sectors are usually simpler in comparison with
those of the 'mother' colonies. This phenomenon corroborates our new proposed
motive, that the new mutants are better fitted to their current growth conditions, but
less adaptable to varying conditions. They are less durable in a dynamic environment
relative to the mother colony, which is less well fitted but more adaptable thanks to
its more complex structure.

(*d*) *Cooperative genetic changes during morphotype transitions*

The *P. dendritiformis* can develop two different morphotypes: simple branching
(SB) and the chiral branching (CB). Each is genetically inheritable: these properties
can be transferred by an individual cell that will develop the same morphotype it
was taken from. We observed spontaneous and induced transitions between the two
morphotypes. Interestingly, the SB → CB transition happens at growth conditions
under which the CB colonies expand faster and exhibit a set of more complex patterns
and vice versa. This phenomenon gives rise to several riddles. One is the manner in
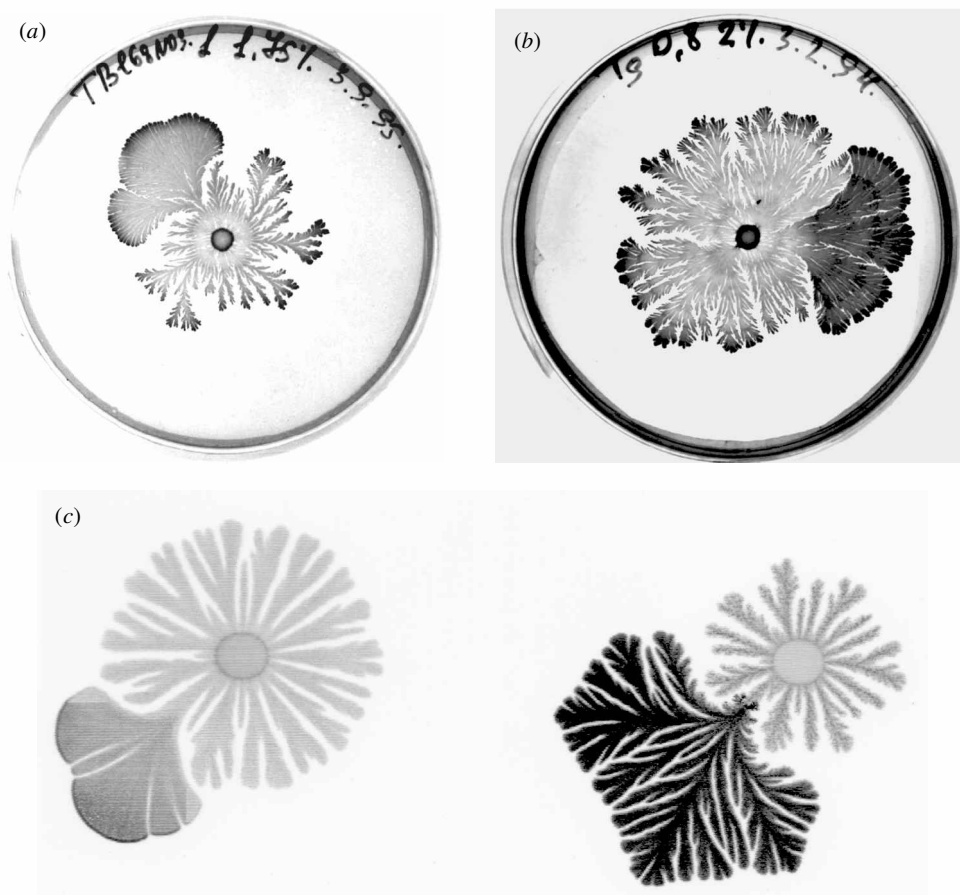which the colony can reach down and induce genetic changes in the individual cells.

Figure 12. Burst of sectors during branching growth: (*a*), (*b*) observations and (*c*) simulations. The two simulations are for mutants with different advantages: higher growth rate (right) and higher sensitivity to repulsive chemotactic signalling (left). In the former case, the sector is not a segregated area and contains both wild-type and mutant bacteria.

Another related riddle has to do with the morphotype bursts. Using our models, we came to realize that sparse long cells scattered among short ones have no advantage. In fact, their movement is far less efficient. Only a finite group (finite nucleation) of long cells can lead to a burst of the chiral morphotype in a manner analogous to phase transitions (say, from liquid to solid) via finite nucleation. Thus, we had to devise a cooperative mechanism whereby many cells are genetically transformed together. We proposed that a possible mechanism is that of autocatalytic genetic change (Ben-Jacob *et al*. 1994*b*). Recent work seems to promise experimental support to this theoretically predicted mechanism.

(*e*) *What lies ahead?*

We saw how the study of bacterial self-organization can benefit from the merger of modelling assistance power with experimental observations and knowledge about general biotic motives. At this stage, modelling efforts are already making significant

progress in providing a framework for linking disparate observations in biotic systems. This is a crucial stage, but the more challenging task is to include in the models the feedback between the genetic information and the collective colonial biophysical and biochemical behaviour: the colonial regulation of the genome dynamics. This will require unravelling the underlying motives common to self-organization of organisms and of their genome and mutual influence.

5. Clues and percepts drawn from bacterial self-organization

(a) *From universality to biotic generality*

Universal principles and unified theoretical frameworks are much coveted in physics and chemistry (Weinberg 1992, 2001). In the life sciences, the goal is usually to elicit the special features of the specific organism under study. Many common phenomena are observed in a wide range of organisms, but are not perceived as universal principles connected by a unifying theoretical framework. Chemotactic response is an example of a widespread phenomenon that has endured over evolution. Response above threshold, autocatalytic emission of chemicals, chemical communication and flagella-propelled motion are additional examples of such phenomena, which can be viewed as general motives extending from cells to organisms.

Other seemingly unrelated biological phenomena could be manifestations of common underlying principles. For example, gene activation–deactivation, excitatory–inhibitory neurons and repulsive–attractive chemotaxis are different expressions of what can be regarded as one general principle. Yet it should be borne in mind that the biotic general principles and motives differ from the universal principles and fundamental laws of physics (energy conservation, the second law of thermodynamics, etc.) that govern both abiotic and biotic worlds (Deblrück 1986; Schrödinger 1944; Jacob 1993); what we are seeking here is biotic generality, not universality.

(b) *Informative communication and adaptable generators*

Colonial adaptability depends on communication (Nester *et al.* 2001; Caporale 1999; Rosenberg 1999; Joset & Guespin-Michel 1993). The communication induces changes in cell behaviour by direct biochemical pathways or by triggering internal self-organization. Sometimes, transfer of genetic material, such as plasmids and phages, can even cause the cell to alter its DNA sequence. It is clear that bacterial communication is inducive, but it can also be informative (Ledoux 1971). By inducive I mean that the absorbed message, say a chemical agent, triggers a specific, predetermined pathway within the receiving cell (Nester *et al.* 2001; Caporale 1999; Rosenberg 1999; Joset & Guespin-Michel 1993; Shapiro & Dworkin 1997; Shapiro 1988, 1995; Albrecht-Buehler 1990; Losick & Kaiser 1993, 1997; Ben-Jacob 1997; Ben-Jacob *et al.* 2000a; Bassler 2002). This pathway, in turn, induces an automatic, predetermined response of the cell. By informative I mean that the message initiates in the receiving cell an individual interpretation process, involving internal restructuring (self-organization) according to its current internal state and previously accumulated information. The new structure affords the cell the freedom to select its response to the message. Such freedom implies that the internal self-organization is connected with generation of new information (Ben-Jacob 1998). By its very nature,

self-organization reflects the system's freedom of response, which is not predetermined.

The phenomena involved in bacterial self-organization, such as morphotype transitions, response to antibiotic stress, myxobacteria life cycle and sporulation, are all evidential of the existence of informative communication. Sporulation, for example, will begin only after consolidation and assessment of the colonial stress by the individual bacteria (Bassler 2002). Simply put, starved cells send a chemical signal conveying their stress. Each of the other bacteria uses the information to 'interpret' the state of the colony relative to its own, and accordingly signals its peers to sporulate or not to sporulate. When all members of the colony have sent out their signal and read all the others', if the 'majority vote' is pro-sporulation, cells do sporulate. This complicated process of internal self-organization results in turning an active cell into a sedentary, long-enduring spore.

The notion of informative communication corresponds to my view of bacteria as 'adaptable generators', that is, they alter themselves according to information received from the environment and send signals to bring about (generate) changes in other bacteria.

(c) From 'more is different' to regulated freedom at all levels

The article 'More is different' (Anderson 1972) presents the view that in the hierarchical organization of nature each level is independent, to a certain degree, of the others, so '...entirely new laws, concepts and generalizations are necessary, requiring inspiration and creativity to such a great degree as in the previous one'. Therefore, it argues, the study of statistical physics is as fundamental as that of atomic physics or that the study of biology is as fundamental as the study of chemistry. Biology should not be perceived as applied chemistry or physics, it says. Chemistry is necessary but cannot be sufficient for understanding biotic systems, as new qualities are generated when molecules assemble into these systems. According to 'More is different', there is no backward influence from the higher levels to the lower. So an understanding of biotic systems is not necessary for understanding the principles of chemistry, as the molecules do not assume new qualities and obey new principles when organizing into a biotic system. The contemporary view of adaptive complex systems, still inspired by 'More is different', is that of 'complexity-based top-level emergence'. When large numbers of elements are assembled and linked to form a complex system, new qualities and properties of the system as a whole, but not of its parts, emerge (Horgan 1995; Gell-Mann 1994; Waldrop 1993; Badii & Politi 1997; Goldenfeld & Kadanof 1999; Vicsek 2001; Langton 1989). Bacterial self-organization holds clues to a new perspective of 'More is different' when applied to biotic systems. The notion of adaptable generators reflects the freedom that elements at the lower level have: to assume new properties and qualities when self-organizing to form the higher level. 'More is different' becomes 'More is different at all levels' when applied to the biotic world. Each level (genome, bacterium, modules such as vortices, up to the whole colony) assumes new traits and informatic abilities as the colony organizes. Informatic abilities include informative communication, the ability to obtain information, information storage, information processing and the generation of new information: all essential biotic motives affording the information-based freedom that distinguishes living from inanimate matter. Hence, it is implied that the knowledge

gathered from the study of isolated bacteria is necessary, but can never be suffi-cient for understanding bacterial cooperative behaviour. Similarly, the study of the cooperative behaviour is necessary for full understanding of isolated bacteria, includ-ing its gene-network structure and dynamics.

Regulated freedom means that the communication biases the cell towards a pre-ferred range, not strictly defined, of possible responses. Thus, cells may 'misbehave' and give rise to features destructive to the colony. This destructive ability of each individual is regulated by the colony. It seems that regulation and freedom are com-petitive. However, the appropriate colonial organization can lead to an increase in both freedom *and* cooperation. This can be achieved by amplification of the infor-mative communication and the generation of a more complex colonial structure.

(d) *Linking colonial informative communication and endurability*

There is another advantage to cooperative complexification. It gives the colony the plasticity it needs to develop higher tolerance and robustness when facing local, relatively weak spatio-temporal perturbations. Higher complexity elevates the colo-nial plasticity and flexibility (tolerance and robustness) with respect to environmen-tal spatio-temporal variations. The plasticity and flexibility impart tolerance and robustness to the colony. Therefore, biotic self-complexification affords better adapt-ability and endurability. To better understand this linkage, we must first re-examine two basic notions: complexity and the environment.

(e) *Structural, operational and generative complexity*

It is widely accepted that self-organization and complexity are closely related. However, complexity is still a blurred intuitive concept with no agreed-upon defini-tion (Horgan 1995). This state of affairs probably stems from common use of the term for different notions. I proposed to distinguish between structural (configura-tional) complexity and operational (functional) complexity in the context of self-organization (Ben-Jacob 1997). Structural complexity refers to the observed spatio-temporal structural variations, both within each level and between levels. Entirely disordered structures and strictly regular ones alike have low structural complex-ity (Ben-Jacob & Levine 2001). Self-similar patterns are the simplest of hierarchical structures (Goldenfeld & Kadanof 1999). Colonies of *P. vortex* bacteria provide an excellent example of high structural complexity. The operational complexity repre-sents the singular interplay between levels. It refers to the structural variations the levels can impose or generate on one another. Theses concepts are valid both for abiotic and biotic self-organization.

Structural complexity is related to colonial plasticity, and operational complexity is related to colonial flexibility. Higher operational complexity expresses the ability to form higher structural complexity; the morphology actually formed depends on the imposed growth conditions. This is demonstrated, for example, by the organizational change in the presence of antibiotics.

The motive of regulated freedom leads to an additional concept of complexity specific to the biotic world: generative complexity. It expresses the ability of the bacteria to generate different morphotypes and the freedom-cooperation interplay of each morphotype. I emphasize that this concept does not describe a specific mode of bacterial self-organization but rather the self-organizational potential of the strains

to deal with a dynamic environment. The bacteria possess the ability to change themselves into the suitable morphotype for each range of environmental conditions, the morphotype that allows them higher colonial complexification (structural and operational) in the given environment. The two possible morphotypes, branching and chiral, of the *P. dendritiformis* bacteria are a manifestation of the generative complexity of this bacterial strain. Myxobacteria's life cycle reflects even higher generative complexity. In this regard, higher generative complexity also provides higher adaptability and endurability of the strain.

(*f*) *Learning from the environment and altering it*

The intricate bacteria–environment relations are an essential element of bacterial self-organization. Not only can the environment affect the colony, but also the bacteria can alter the environment for their own benefit. The colony can 'read' the environment and actively probe it (Nester *et al.* 2001; Caporale 1999; Rosenberg 1999; Joset & Guespin-Michel 1993). A thought-provoking example is provided by the myxobacteria (Nester *et al.* 2001; Caporale 1999; Rosenberg 1984, 1999; Joset & Guespin-Michel 1993; Shapiro & Dworkin 1997; Kuner & Kaiser 1982; Dworkin 1999). When food is depleted, the colony generates and sends out 'foraging agents', which are moving groups of adventurous cells (as opposed to social cells) (Shapiro & Dworkin 1997). These foraging agents can send out signals to detect targets far away. When a target is detected, they move towards it and engulf it for investigation. They signal their findings back to the colony.

Often the environment is composed of biotic elements, or an organism. The bacterial interplay with a biotic environment has all the features of the interplay between the colony's internal levels discussed earlier. Through communication, the bacteria can obtain information about the organism that serves as their environment and can alter themselves and that organism accordingly, for their own benefit. The above is a reflection of the biotic generality of motives and principles that is essential for consistency between interacting biotic systems.

Regulation and the induction of beneficial changes in an abiotic environment are manifested, for example, in the way that bacteria altered the Earth's climate. Less evident, and not yet commonly recognized, is bacteria's ability to obtain information from the environment and process it in the same manner that they do informative messages from biotic systems. It must be realized that the abiotic environment can also contain useful available information for the bacteria.

In many experiments, bacteria are exposed to lethal constraints in order to select mutants that happen to have the appropriate trait for surviving. The selective conditions are conceived as an imitation of the environmental action in natural selection. Usually, the effect of one specific selection factor is tested under uniform and constant conditions. The above-described approach is well developed and provides an efficient test bed for studying issues related to the selection for which they were designed. It is not suitable, however, for revealing the significant continuous role of the environment in bacterial 'self-improvement' between the rare events of selection, due to a large sudden change in a specific factor.

The natural environment constantly provides spatio-temporal variations of different magnitude. Colonial complexity is advantageous for endurability in such a dynamic environment, but is not the best adaptation to a static environment with a

specific stress factor. Moreover, a colony that will develop the best structural adaptation for a specific environment will not be flexible enough to endure changes in this environment (Delbrück 1946), as seen, for example, in the bursts of sectors discussed in § 3.

A dynamic environment, unlike a static one, does contain information. That information, however, is distributed over space and time, while each bacterium has the capability to read, process and store local information only. Information about spatial variations can be communicated throughout the colony. To obtain information about temporal variations over a long duration, inheritable memory is required. Cell differentiation and inherited gene expression are examples of genetic memory. I proposed that colonial-aided self-executed genetic changes, especially in the non-coding part of the DNA, might provide an additional mechanism for long-term memory (Ben-Jacob 1998). The colony can use these abilities together with the informatic capabilities and regulated freedom at each of its levels to 'learn from the environment' and 'improve itself accordingly'. By learning, I mean the collective colonial information processing. The colony continuously alters itself in order to perform the computation and according to their outcome. Thus, as a computation unit, it is not limited to the abilities of the universal Turing machine (Siegelmann 1995; Atlan 1987) but can both generate and 'interpret' information.

The bacteria manipulate the environment for their own benefit. They can even trigger a self-organizing process in the environment and use the newly produced information for further self-complexification: a process that could be viewed as autocatalytic complexification. An illuminating example is the bacterial generation of convection for a better supply of oxygen (Kessler & Wojciechowski 1997; Kessler & Hill 1997).

6. The hypothesis of co-enhancement of complexification, adaptability and endurability

My hypothesis is that the informative communication between individuals enhances the individuals' regulated freedom, and at the same time intensifies their cooperation. This apparently paradoxical process is accomplished via cooperative complexification of the colony through self-organization of hierarchical spatio-temporal patterning. The colonial higher complexity provides the degree of plasticity and flexibility required for better colonial adaptability and endurability in a dynamic environment. The biotic system can modify the environment and obtain environmental information for further self-improvement by autocatalytic complexification.

(*a*) *A proposed definition of biotic self-organization*

With the new picture in mind, I propose the following definition for biotic self-organization: co-generation, based on informative communication, of cooperative complexification and regulated freedom at all levels—from the genome of the individuals to the whole system—manifested in the formation of flexible complex hierarchical spatio-temporal patterning for improved adaptability and endurability of the system in a dynamic environment.

(*b*) *From intuitive to measurable concepts*

Previous attempts to devise a general theoretical framework for self-organization (e.g. dissipative structures, catastrophe theory and synergetics) did not yield testable theories, perhaps because the new concepts involved were not assigned measurable definitions.

My proposed picture presents a long list of new intuitive concepts, all linked to the notion of structural complexity. To convert this hypothesis into a testable theory, the first step is to turn the intuitive concept of structural complexity into an operational definition. We have recently accomplished this task (Hulata *et al.* 2003), and it can now provide a basis for measurable definitions of the other concepts.

(*c*) *Experimental support*

The above concepts were conceived in view of the body of existing (cited) knowledge and observations. Additional experiments we performed that are not described above include the following.

(i) Self-organization with imposed geometrical modulation of the surface, to demonstrate colonial plasticity (Ben-Jacob 1997).

(ii) Self-organization of *P. dendritiformis* in the presence of antibiotics; according to the growth conditions, the morphotype with the higher complexity exhibits lower sensitivity (higher flexibility) compared with the other, although the individual isolated bacteria of both morphotypes have similar sensitivity.

(iii) Induced transitions between these morphotypes by designed (engineered) structuring of the environment (figure 13). These experiments demonstrate the 'generative, complexity-based adaptability' and bring forward the new applicable idea of 'engineered self organization', or designing the environment to guide self-organization in the direction of desired features.

(iv) Comparison of colonial growth on minimal agar (adverse but 'simple' environments) and on peptone (less adverse and more 'complex') reveals patterning that is more complex in the second case.

(v) Testing bacterial effect on the environment is usually more challenging. It is transparent in the case of bioconvection (Kessler & Wojciechowski 1997; Kessler & Hill 1997). We did some indirect observations of induced patterns on solid substrates, which also seem to support the idea. An even greater challenge is to demonstrate the notion of autocatalytic complexification. In the last decade, we 'played' in the lab with engineered micro-evolution over several years. We managed to have the original strains evolve into new ones capable of generating patterns far more complex and on a wider range of growth conditions (Ben-Jacob *et al.* 1997*a*).

The above are just preliminary results. Many more, intensive experimental studies should be performed within the new approach, with dynamic and engineered environments instead of selective homogeneous ones.
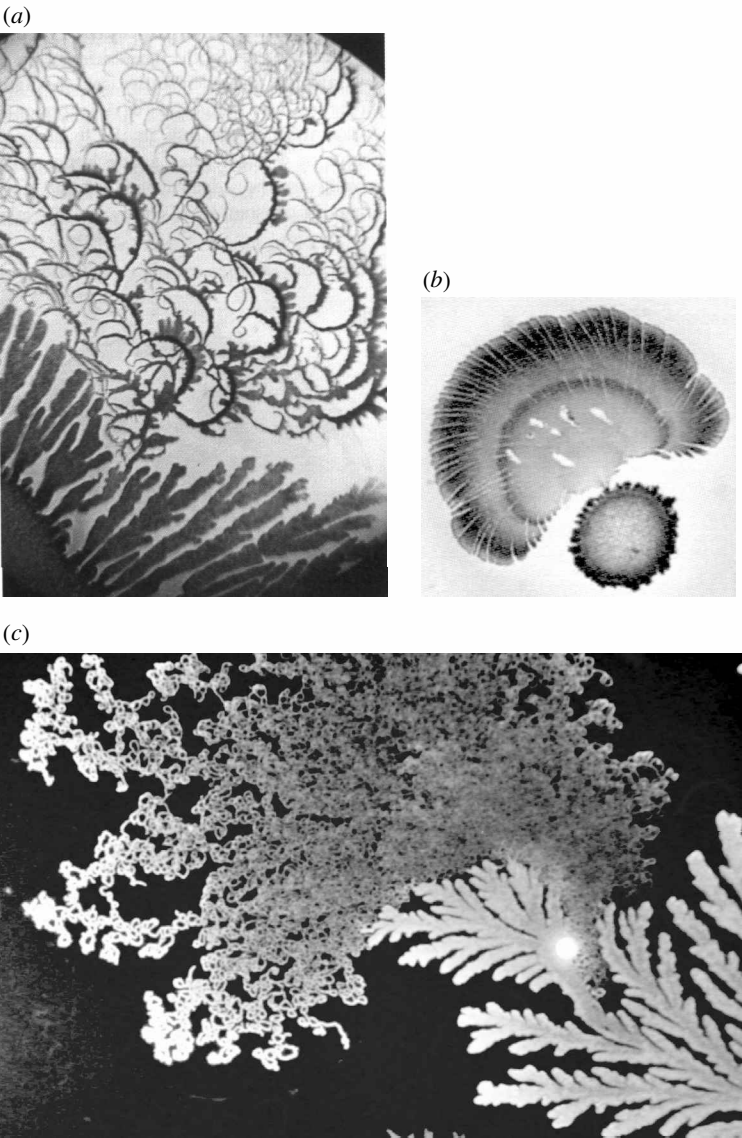
(a)

(b)

(c)

Figure 13. Morphotype transitions between branching and chiral. (*a*) Spontaneous transition into chiral morphotype during growth on soft substrate. (*b*) The spontaneous transition is from chiral back to tip splitting during growth on hard substrates. In both cases, the transition is into the faster and more complex morphotype for the given growth conditions. (*c*) Induced transition into the chiral morphotype by 'biotic perturbation' (fungi located at the bright spot). The higher flexibility of the chiral morphotype enables it to cope better with the perturbation. Note that it changes its ordinary geometrical organization to deal with the perturbation, a change possible thanks to its higher flexibility.

(*d*) *Reflections on abiotic self-organization*

One of the important lessons we learned from the bacteria is that the nature of the environment (e.g. 'simple' and adverse versus 'complex') and its effect on self-
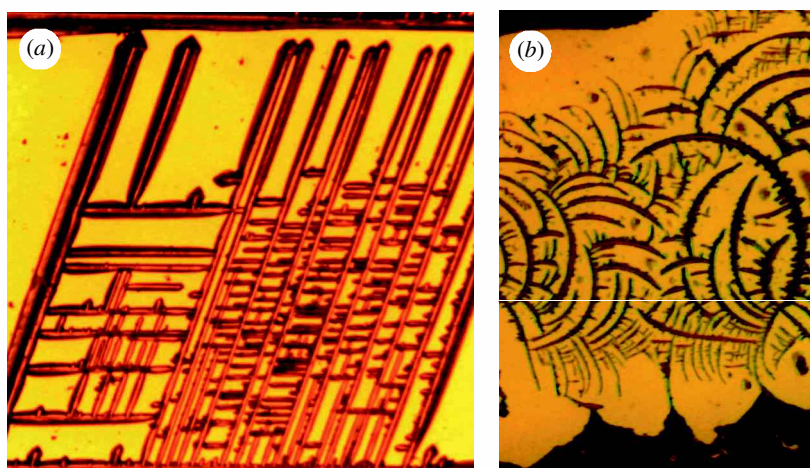
Figure 14. 'Engineered self-organization' in solidification from supersaturated solution (*a*) at *ca.* 28% NH$_4$Cl and 0.2% CuSO$_4$ and (*b*) during drying of the bacterial substrate, containing left-handed and right-handed chiral molecules. In (*a*), the growth has been 'engineered' to start from a long bar. It demonstrates the singular effect of the CuSO$_4$ that leads to the emission of side branches at an angle of 72° (corresponding to 10-fold symmetry) instead of the 90° of pure NH$_4$Cl. In (*b*), corrugated boundaries helped to produce the observed pattern.

organization should be reconsidered. For example, in Hele-Shaw experiments, when water is pressurized to invade glycerin, a branching pattern forms. The applied pressure is perceived to be the 'driving force' or the system's 'distance from equilibrium'. Higher pressure usually leads to more complex-looking patterns. In this experiment, the fluids are constrained between two plates. Taking out the top plate and letting the water 'freely' invade the glycerin results in far more complex spatio-temporal behaviour. Complex as it is, it can be further complexified by adding food colouring to the glycerin (video available from the author upon request). Another example of the effect of the environment on the self-organization is shown in figure 14. In this case, we pre-designed the conditions to obtain a network structure, one of straight bars, with side branches shooting out at an angle of 72°, and the other of curved branches with alternating handedness (Ben-Jacob 1993; Raz *et al.* 1991).

At present, the notions of the 'driving force' and the 'distance from equilibrium' of a system are commonly accepted as well defined and unquestioned. Reflecting from bacterial studies, I suggest that they should be rethought and redefined to include the interplay between system and environment from a different perspective.

(*e*) *Engineered self-organization of systems too complex to design*

With the rapid developments in nanotechnology, it seems that production of molecular electronic components in any desired number will soon be attained. However, the realization is now starting to dawn that nobody knows how to assemble a huge number (say 10^{11}–10^{14}) of elements into a functioning system.† The engineered self-organization of biotic and abiotic systems and the idea that complexification requires elements with regulated freedom suggest that the barrier can be overcome

† This point was raised by D. Haviland at the symposium.

by an approach of 'engineered self-organization of adaptable components'. These components should be pre-designed to have a flexible internal structure, which they can change, and informatic capabilities. Many collections of such elements will be let to self-organize in a pre-engineered environment they can exchange information with. The most efficient results will be allowed to self-improve further. The self-improvement will involve genetic algorithm-like evolution of the components' internal structure and informatic capabilities (the analogue of evolution of the potential for gene expression in biology). The system itself will regulate this evolution of its components.

As brought up by Haviland during the symposium, one criterion for the maturity of a new field is its ability to suggest new applications. Engineered self-organization of systems too complex to design seems to fill this requirement.

7. Epilogue

My hypothesis of co-enhancement of regulated freedom and cooperative complexification might provide a connection between neuronal plasticity and the emergence of cognition, as it implies that higher complexity requires higher plasticity (Skoyles & Sagan 2002). Neuronal plasticity can be viewed as the regulated freedom at all levels, from the neuronal genome through neurons and neuronal networks and up to the central nervous system. All these levels fit the notion of adaptable generators with informatic capabilities. Currently, cognition is viewed as a top-level emergent phenomenon. According to my new hypothesis, cognition is a reflection of regulated freedom, so that cognition of the system as a whole has to be self-generated together with some degree of cognition (freedom) at every level.

Two major events in Earth's history bear the mark of self-organization: the emergence of life from inanimate matter and the generation of cognitive abilities in living creatures. Bacteria, having originated in the first event and been a link in the second, are nature's bridge between inanimate matter and cognition. Perhaps they might shed some light on both events.

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