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The Origin of Life: Chemical Evolution of a Metabolic System in a Mineral Honeycomb?

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Abstract For the RNA-world hypothesis to be ecologically feasible, selection mechanisms acting on replicator communities need to be invoked and the corresponding scenarios of molecular evolution specified. Complementing our previous models of chemical evolution on mineral surfaces, in which selection was the consequence of the limited mobility of macromolecules attached to the surface, here we offer an alternative realization of prebiotic group-level selection: the physical encapsulation of local replicator communities into the pores of the mineral substrate. Based on cellular automaton simulations we argue that the effect of group selection in a mineral honeycomb could have been efficient enough to keep prebiotic ribozymes of different specificities and replication rates coexistent, and their metabolic cooperation protected from extensive molecular parasitism. We suggest that mutants of the mild parasites persistent in the metabolic system can acquire useful functions such as replicase activity or the production of membrane components, thus opening the way for the evolution of the first autonomous protocells on Earth.

Keywords RNA world · Mineral matrix · Replicator evolution · Inorganic compartments · Origin of life

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Introduction

During the past two decades, the RNA-world hypothesis (Gilbert 1986; Joyce 2002; Orgel 2004) has become the dominant paradigm of research on prebiotic evolution. The reason for this is that RNA or RNA-like macromolecules may have naturally played the dual role of *genes* and *metabolic enzymes* in primordial forms of life, with their genotypic and phenotypic functions directly linked to each other. The RNA-world hypothesis was based on some earlier empirical discoveries, suggesting that certain RNA molecules called ribozymes often perform catalytic activities of different sorts (Guerrier-Takada et al. 1983; Kruger et al. 1982) that might be useful or even necessary for the survival of the cells possessing them. The essential catalytic role of RNA in basic biological processes like transcription and translation has been clearly demonstrated since then. Particularly well studied are the RNA molecules playing a crucial role in ribosome structure and function (Moore and Steitz 2002; Steitz and Moore 2003). More recently, the possibility of RNA self-replication and exponential population growth without any protein-based catalytic support was proven for the first time in vitro

(Lincoln and Joyce 2009). No simple chemical entity other than RNA has been credibly proven so far to have the potential of carrying the functions of information transmission and catalytic activity simultaneously, and maintaining their definite functional linkage. Therefore, we have no reason to assume that the first prebiotic replicators were very different from recent RNA oligo- and polynucleotides.

Group Selection and the Ecology of the RNA World

The idea that an assembly of RNA-like molecules may have had constituted the first simple evolvable metabolism-template (MT) type infrabiological system (Szathmáry 2006, 2007) has appeared in at least two different scenarios of the origin of life: in the *hypercycle model* (Eigen and Schuster 1977) and in different versions of the *metabolic replicator model* (Czárán and Szathmáry 2000; Scheuring et al. 2003; Szathmáry and Demeter 1987). Assuming that a system of replicators has to admit a certain level of complexity from the start in order to be able to work and evolve, one is faced with the basically ecological problem of keeping the assembly together without the fastest-replicating member outcompete all the rest. The competitive exclusion of replicators carrying vital functions for the system is necessary to avoid in order to maintain both the phenotypic functionality and the genetic information content of the assembly. The hypercycle and the metabolic replicator model postulate different mechanisms for that purpose: The hypercycle assumes a cycle of direct heterocatalytic help among autocatalytic replicators (Fig. 1a), whereas the metabolic model relies on the indirect mutualistic interactions of autocatalytic replicators, through their heterocatalytic contributions to a common metabolism that produces monomers for their own replication (Fig. 1b). It has been shown that in a homogeneous, spatially mixed (nonspatial) setting the hypercycle is exposed to the lethal effect of parasitic sequences of two different kinds (Maynard Smith 1979; Maynard Smith and Szathmáry 1995), and the metabolic replicator model is not viable at all because the mutualistic benefit of metabolism is aspecific in the sense that it helps all the members of the replicator assembly alike. Thus, the fastest-replicating member is still able to exclude all other replicators and the spatially homogeneous metabolic system also collapses (Czárán and Szathmáry 2000; Eigen and Schuster 1979). All known solutions to the problem of the ecological loss of genetic information and phenotypic function are of the same genre: each assumes some level of group selection within a large population of replicators. Group selection may be very effective on definite assemblages of replicators enclosed in some kind of membrane vesicles or *protocells*: the compartmentalized hypercycle and the protocellular stochastic corrector model (Fontanari et al. 2006;

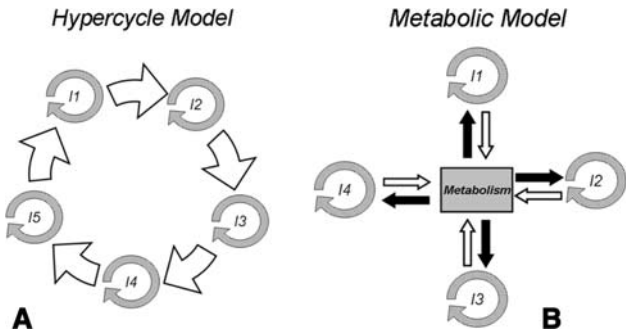

Fig. 1 **a** Hypercycle model. $I_{1..5}$ are autocatalytic replicators; solid circular arrows show replication; arrows represent the heterocatalytic help among replicators. **b** Metabolic model. $I_{1..4}$ are replicators; *filled box* represents metabolism; *black arrows* represent the supply of metabolites (monomers) used for replication; *solid circular arrows* show replication; *white arrows* mean the catalytic effect of metabolic replicators helping metabolism

Szathmáry and Demeter 1987) are both viable and resistant to their parasites, although at high mutation rates (for which the whole coexistence question has been raised anyway) the stochastic corrector model outperforms the compartmentalized hypercycle (Zintzaras et al. 2002). The only snag is with the assumption of the proto-cellular structure in the first place: nothing within these models explains where it comes from and how it is maintained. Therefore, we need a physico-chemically more plausible mechanism of group selection to explain the coexistence and coevolution of a number of otherwise competing replicators.

The Mineral Honeycomb

If the RNA world ever existed, it is unlikely that it had developed in a dilute aqueous solution, due to the difficulties of polymerization and the instability of polymers in such an unstructured environment. It was Bernal who first recognized in his seminal work (Bernal 1951) that certain mineral surfaces could have played a pivotal role in prebiotic chemistry. His hypothesis of a mineral-mediated origin of life has received ample support from both theorists and experimentalists, because it helped to solve some, thus far inaccessible, problems of the RNA-world hypothesis. He assumed that minerals could have favored the very first steps of prebiotic evolution by concentrating reactants on their charged surfaces (Baaske et al. 2007; Koonin 2007), by catalyzing chemical reactions (Biondi et al. 2007a; Costanzo et al. 2007; Ertem and Ferris 1996; Ferris et al. 1996; Ricardo et al. 2004; Saladino et al. 2004) and protecting biologically important molecules from degradation in a chemically hostile environment (Biondi et al. 2007b; Gallori et al. 2006). Now we may add yet another important item to this, already impressive list of advantages: mineral surfaces can also mediate selection for

cooperation through temporarily sustaining local neighborhood structures in replicator assemblages. In previous works (Czárán and Szathmáry 2000; Könnyü et al. 2008), we have explored the potential of mineral surfaces to preserve the genetic diversity and the phenotypic functionality of the metabolic replicator system even in the presence of parasitic sequences, and we also demonstrated that the hypercycle model remains vulnerable to lethal damage by its parasites even in this spatially explicit selection setting (Czárán and Szathmáry 2000; Könnyü et al. 2008; Scheuring et al. 2003).

A closer look at the microscopic structure of mineral surfaces reveals that these are by no means flat—in fact they are a complicated network of interconnected pores and cavities inside the mineral. This might have profound implications on the dynamics of group selection in replicator assemblages: pores offer semi-isolated compartments somewhat similar to proto-cells, which represents a different, more realistic selection regime compared to that on flat surfaces.

Smith and his coworkers have identified *feldspar* as the possible honeycomb for the very first stages of biochemical evolution (Parsons et al. 1998; Smith 1998; Smith et al. 1999). Feldspar is by far the most abundant group of minerals in the earth's crust, forming about 60% of terrestrial rocks crystallize from magma in both intrusive and extrusive igneous rocks; K-feldspars must have been common on the surface of prebiotic Earth $\sim$3.8 Ga. Weathered surfaces of K-feldspars from granitic source rocks and from feldspathic gneisses are complex and covered in regularly distributed etch-pits and grooves. The etch-pits generate extraordinary, cross-linked tubular networks (Fig. 2a), which extend to $\geq$50 μm below the surface (Parsons et al. 1998). On every mm^2 of weathered feldspar surface, there would have been hundreds of catalytic microreactors, open to diffusion but protected from the dispersive effects of flow and convection in a fully open system, and also from ultraviolet radiation. The reactors normal to the surface would have communicated laterally through narrower connecting tubes (Fig. 2b) so that ever more complex polymeric molecules, catalytically assembled on the silica-rich areas on the tube walls, could have spread through the honeycomb zone (Parsons et al. 1998).

Similarly, other authors ascribe a critical role to the 3D porous structure of iron monosulphide precipitates or aragonite ($CaCO_3$) in hydrothermal vent systems (Koonin and Martin 2005; Martin and Russell 2003; Martin and Russell 2007). Recently, the extreme accumulation of nucleotides in simulated hydrothermal pore systems driven by thermodiffusion has been shown. The simulations predict that millimeter-sized pores accumulate RNA mononucleotides and oligonucleotides more than 10^8-fold into micrometer-sized regions. These results suggest that interlinked mineral pores in a thermal gradient may provide a compelling high-concentration starting point for the molecular evolution of life (Baaske et al. 2007; Koonin 2007).

Based on a simplified cellular automaton (CA) representation of such a "porous environment," we have explored the effect of group selection in a network of semi-isolated microreactors on the coexistence and the parasite resistance of a system of metabolically coupled replicators.

Model

The assumptions of the model follow those of the CA described by Czárán and Szathmáry (2000) except that here each cell of the square lattice represents a pore *inside* the mineral, instead of a "site" suitable for the adhesion of a single replicator on the mineral surface. Neighboring pores are interconnected so that limited diffusion of matter through the connecting channels is possible.

Each pore can contain at most s RNA-like replicator molecules and each replicator within a pore belongs to one of I different types, so that

$$\sum_{n=1}^{I} R_{ij}(n) + e_{ij} = s \qquad (1)$$

where $R_{ij}(n)$ is the number of replicators of type n, and e is the number of empty spots available for replicator molecules to occupy in pore ij.

Each of the I replicator types is characterized by a growth constant (k), a decay constant (d), and a specific enzymatic activity with which it contributes to the metabolic activity of the within-pore assembly of replicators. One generation consists of four events in each pore: (1) resource uptake, (2) nucleotide production (metabolism), (3) replication and decay of replicators, and (4) migration (diffusion) of replicators between neighboring pores.

Resource and Monomer Balance, Metabolism, and Storage Within Pores

A pore is considered a stir-flow microreactor externally fed raw materials for nucleotide synthesis. Raw materials ("resources") flow into each pore at a constant rate from an external source like in a "black smoker" (Martin and Russell 2003; Martin and Russell 2007; Parsons et al. 1998).

Metabolism converts raw materials to monomers (nucleotides), the building blocks for later replication. Pores are assigned a "storage capacity" with regard to raw material and locally produced nucleotides: up to a certain concentration resources not converted by metabolism and

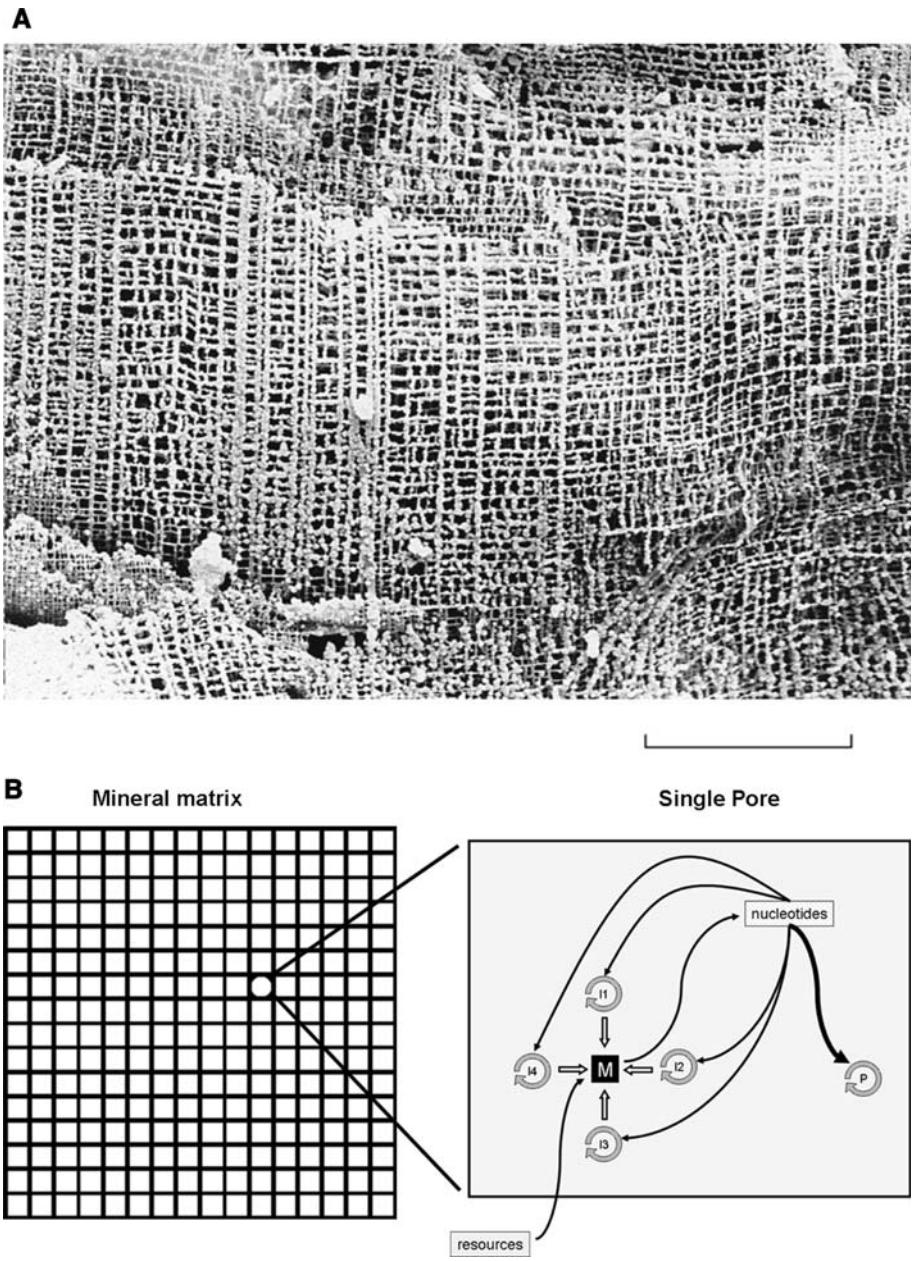

Fig. 2 **a** SEM image of a resin cast of an etch-pit network near the surface of a weathered Shap alkali feldspar (scale bar 20 μm). The cast was made by impregnating the feldspar with Araldite resin under vacuum, curing, and dissolving away the feldspar in concentrated HF. The surface of the feldspar is off the bottom of the micrograph, and the image is of a pile of two-dimensional networks that have fallen over to lie on top of each other. Because the resin is flexible, parts of the networks are curved. The original etch-pits were developed on edge dislocations very nearly parallel to b (*horizontal*) and c (*vertical*) in the perthite contact plane close to 601 of the monoclinic feldspar (SEM picture and caption from Fig. 2 of Parsons et al. 1998). **b** $I_{1..4}$ are the metabolic replicators; M is metabolism. *Solid arrows* represent the flux of resources (raw materials) outside the pores chemically transformed inside the pores in nucleotides by the catalytic activity of replicators (ribozyme). *White arrows* mean the catalytic effect of metabolic replicators helping metabolism. P represents a parasitic replicator that uses the monomers supplied by metabolism, but it does not help producing them

nucleotides not used up by replications remain available for later use. Thus, at the beginning of generation t, the quantity r_{ij} of resource available for metabolism in pore ij is.

$$\begin{array}{ll} r_{ij} = r_{ij}(t-1) + J(t) & \text{if } r_{ij}(t-1) + J(t) < r_{\max} \\ r_{ij} = r_{\max} & \text{if } r_{ij}(t-1) + J(t) \geq r_{\max} \end{array}, \qquad (2)$$

where $J(t)$ is the influx of raw materials and $r_{\max}$ is the storage capacity of a pore with respect to the resource.

The nucleotide monomers for replication are produced by the RNA-like replicator enzymes themselves inside the pore from the resource, provided all the necessary enzyme activities are present and operate a simple network of chemical reactions constituting "metabolism" (Fig. 1b). Each replicator within the pore contributes to the metabolism of the pore by catalyzing a single reaction of the metabolic reaction network. Metabolism is "minimal" in the sense that with any one of its enzymes lacking there is no alternative way of producing monomers for replication: the pore with an incomplete set of metabolic enzymes is metabolically "dead." To quantify the metabolic "fitness" M_{ij} of a pore, i.e., the efficiency of the conversion of raw materials to monomers within, we use the same metabolic function as in Czárán and Szathmáry (2000):

$$M_{ij} = \left(\prod_{n=1}^{I} R_{ij}(n) \right)^{I^{-1}} \frac{I}{s}. \quad (3)$$

M_{ij} is the geometric mean of the within-pore copy numbers $R(n)$ of all replicator types contributing to metabolism, scaled into the (0, 1) interval by the normalizing factor I/s. Note that M_{ij} is zero if any one of the metabolic enzymes is missing from the pore, and it attains its maximum (1.0) in a pore saturated with an even distribution of metabolically active replicators.

The speed at which resources are converted to monomers is proportional to the amount of resources r_{ij} present and the metabolic efficiency M_{ij} of the pore, so that the actual amount of nucleotides, m_{ij}, at the disposal of local replication within pore ij at generation t is

$$\begin{array}{ll} m_{ij}(t) = m_{ij}(t-1) + c \cdot M_{ij} \cdot r_{ij} & \text{if } m_{ij}(t-1) + c \cdot M_{ij} \cdot r_{ij} < m_{\max} \\ m_{ij}(t) = m_{\max} & \text{if } m_{ij}(t-1) + c \cdot M_{ij} \cdot \mathrm{res}_{ij} \geq m_{\max} \end{array}, \quad (4)$$

where c is a proportionality constant and $m_{\max}$ is the storage capacity of a pore with respect to the monomers.

Replication and Decay

Given at least one replicator, a sufficient amount of monomers, and empty sites to accommodate new copies of replicators within the pore, local replication is possible. If any one of the prerequisites (at least one replication unit of nucleotides and at least one empty site) is missing, replication does not occur even if there are replicators present in the pore. If conditions for replication are met, then each resident replicator has a chance to delegate an offspring of its own into the empty site. Which of the candidates actually succeeds is determined by a draw as follows: each of the I replicator types has a claim to occupy the site. The claim of type n is

$$C(n) = k(n) \cdot R(n), \quad (5)$$

where $k(n)$ is the replication constant and $R(n)$ is the within-pore copy number of type n replicators. The probability that it will be type n that wins the game for the empty site is

$$P(n) = \frac{C(n)}{\sum_{x=1}^{I} C(x) + C_e}, \quad (6)$$

in which C_e is the claim constant of the empty site for remaining empty. Obviously, the chance that the site remains empty despite the presence of able candidates for occupying it is

$$P(e) = \frac{C_e}{\sum_{x=1}^{I} C(x) + Cl_e}. \quad (7)$$

During each replication event, both the amount of nucleotides available for further replication and the number of empty sites decrease by one unit. The replication cycle is reiterated within the pore until either the nucleotide concentration or the number of empty sites shrinks below one, or else if all the replicators that were present in the grid at the beginning of the generation have already been replicated once. Of course we assume that the replication events are simultaneous, and each replicator has only one chance to replicate within a generation time, because replication is the slowest of all processes in the model. Generations are defined by the time scale of a single replication.

The replication phase is followed by a round of replicator decay: each replicator present in the pore disappears by a type-specific probability $d(n)$. Decay may be due to chemical (breaking of the macromolecules) or physical (washing them off from the walls of the pore) processes.

Replicator Migration (Diffusion)

Due to the interplay of adhesive and (mainly thermal) dissociative forces between the replicator macromolecules and the walls of the pores, the replicators are assumed to "crawl" within pores, and, using the channels connecting them, sometimes also between neighboring pores. Migration farther away than a first-neighbor pore is not allowed in one generation time. Within-pore movement involves just a site change inside the pore, the claim of which event for occurring, $Q_{ij,ij}$, depends on the proportion of empty (e) sites available within the corresponding pore:

$$Q_{ij,ij} = \frac{e_{ij}}{s}. \quad (8)$$

The claim $Q_{ij,xy}$ of the event that a replicator resident in pore ij migrates to one of the neighboring pores xy depends on the proportion of available empty sites in xy and the migration constant d:

$$Q_{ij,xy} = d \cdot \frac{e_{xy}}{s}. \quad (9)$$

Thus, the probability that a replicator in pore ij will end up in xy, a pore within its own neighborhood by the end of the generation is

$$P_{ij,xy} = \frac{Q_{ij,xy}}{\sum_{vz} Q_{ij,vz}}, \quad (10)$$

where xy and vz run through all the pores within the neighborhood of ij, including ij itself. Internal migration corresponds to no migration on the pore level, of course, as we do not keep track of within-pore spatial positions. The migration procedure is run once on the average for each replicator present in the system using a random update routine at the end of each generation.

Metabolic Parasites

Like any community of cooperating entities sharing the benefits of cooperation, the metabolic system is also exposed to the attack of parasites which take the benefits, but avoid the burden, of cooperation. The sole conceivable type of opportunistic pest for the metabolic system is a replicator that taps the output of metabolism (monomers) by using up the yield without itself actually contributing to production (Fig. 1b). We have introduced two variants of this parasite into the metabolic pore model: a fast one with a replication constant much higher than that of the fastest cooperating replicator ($f = 20.0$) and a moderate one that has a replication constant equal to that of the fastest cooperator ($f = 8.00$). The parasites were released at random within the grid, using a series of different invasion rates.

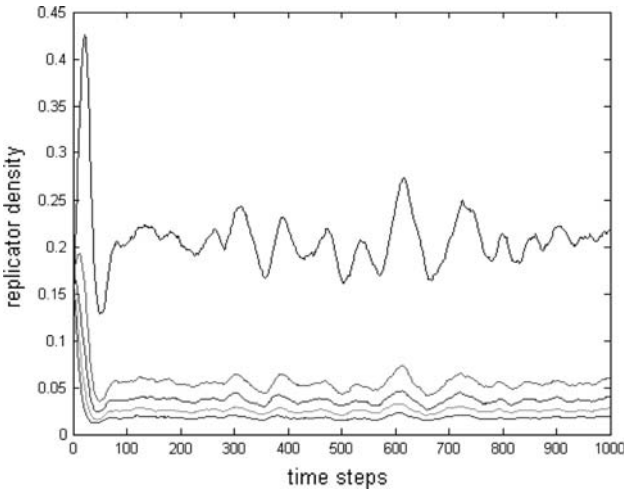

Fig. 3 Population dynamics of five coexisting replicators. *Fixed parameters*: diffusion parameter (d) = 0.02; pore size (s) = 40; resource = 2. The different replicators have different growth constant from top to the bottom $K_i = 8, 6, 5, 4, 3$, respectively. Each time step represents a single generation as defined in the model section

Results

The spatially homogeneous (mean-field) approximation (cf. Czárán and Szathmáry 2000) of within-pore dynamics predicts that the replicator population of a single, isolated pore is doomed to extinction for two reasons. First, if the pore harbors a large number of replicators, then the types of higher replication constants will eventually outcompete the slowest type and—thereby lacking a complete metabolic set at their disposal—die out too. The competitive pressure remains the strongest on the slowest replicator until it goes extinct because the benefits of metabolism are aspecific: all replicators exploit the same pool of monomers which is equally available to all of them. What is more, if the total population is small, then on top of the competitive effect even stochastic drift might exterminate one of the replicator types, and the result is even worse than with large populations.

Coexistence and Persistence

Coupling the pores by—even a slight—migration of the replicators changes the dynamics dramatically, so that it maintains coexistence as shown in Fig. 3. The coupled system has been initiated by an even distribution of five different replicator populations scattered at random over half of the sites available. This is an ideal start for population growth, with a sufficiently high number of available sites in each pore and a rich supply of monomers delivered by metabolism working at its optimum—the reasons for all replicators multiplying fast at the initial phase of the simulation. Due to differences in replication constants, the ideal distribution of metabolic enzymes soon starts to deteriorate everywhere, which obviously slows down replication for shortage of monomers. This in turn results in a relatively low, yet still nonzero average density of replicators in the long run: overall replicator abundance approaches a quasi-stationary state with somewhat irregular oscillations. The spatial image of population densities (Fig. 4) becomes clustered and shows a traveling wave pattern in time. This is to be expected because the ideal circumstance for a competitively inferior (small k) replicator to survive is to spread to unsaturated "habitats" (pores) in order to escape severe competition from faster-replicating types. These "refugia" themselves become saturated soon, however, and the slow replicator species has to move on again if it is to survive. For this reason, it is necessary that a patch of viable pores (i.e., those containing metabolically complete replicator sets) be surrounded with a sufficient number of empty pores to ensure refugia to the competitively inferior species at all times. Wherever a metabolically active patch grows too large, its central region suffers a metabolic breakdown due to the competitive exclusion of at least one subordinated replicator species in the absence of an unsaturated (empty) refugium within its reach. Thus, the large patch breaks up into small ones again, and each small patch starts growing until it becomes too large again, and so on. This is the equivalent of an excitable medium scenario with a refractory phase (Greenberg and Hastings 1978) obviously resulting in the well-known spatiotemporal pattern of traveling and interfering waves. Empty pores represent the excitable phase, metabolically active pores are the excited ones, and the refractory phase includes metabolically inactive but saturated pores.

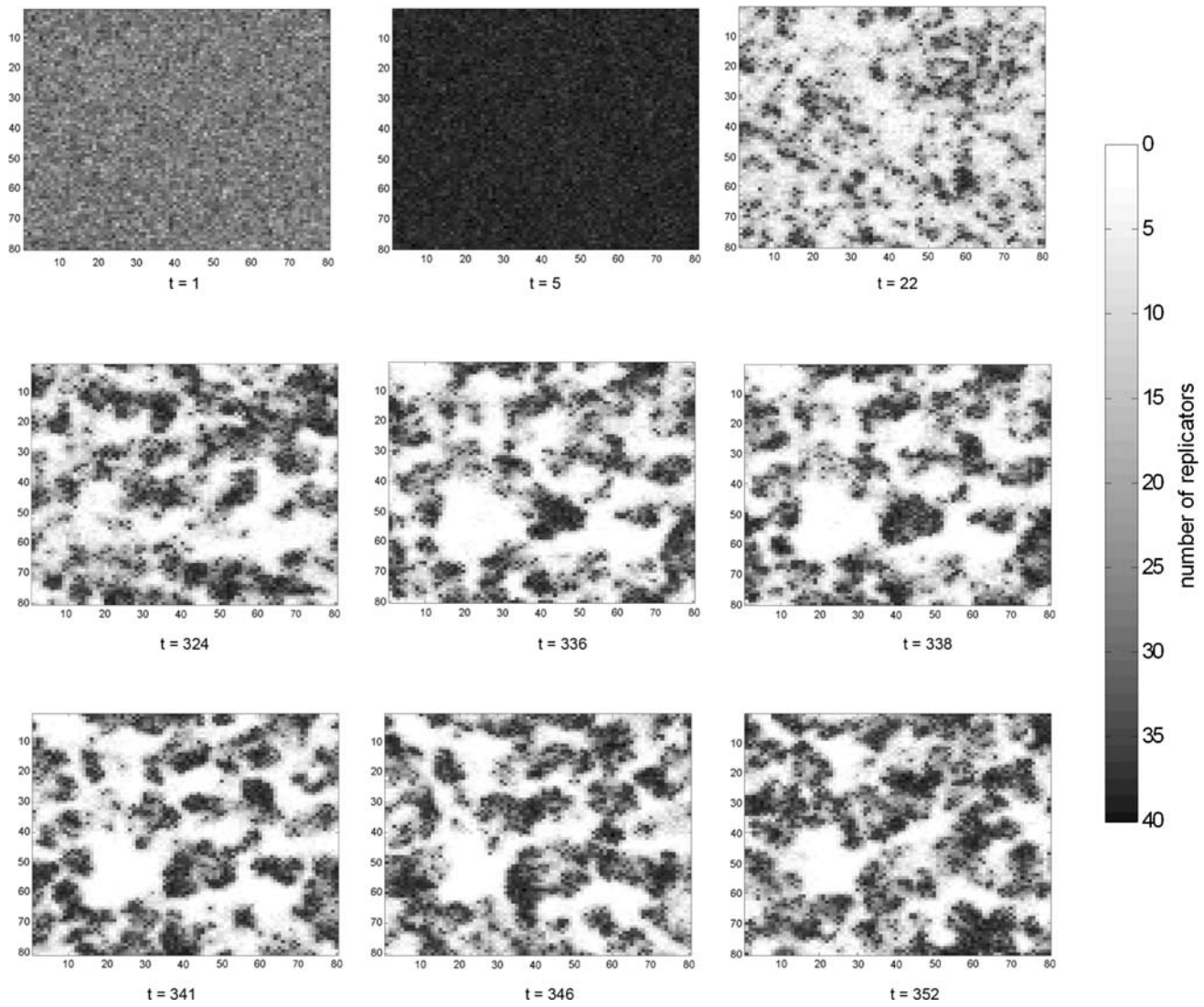

Fig. 4 Spatial representation of the pore system. Each cell in the grid represents a pore; replicator abundances are labeled in grayscale. The parameters are the same as in Fig. 3

How does the coexistence (and thus the persistence) of the metabolic system in a network of pores depend on the crucial parameters of the model? The answer to this question has been summarized on the graphs of Figs. 5 and 6. We consider the migration parameter d, the number of different replicator types contributing to metabolism (system size, nr), and the maximum number of replicators per pore (pore size, s), as the crucial parameters of our model—we discuss the effects of changes in these in turn below.

d is the "drive" of the replicators to emigrate: if it is 0, the replicators do not leave the pores of their actual residence. $d = 1$ means no preference toward the residential pore: each empty site within the whole neighborhood is equally available for the replicator in the focal pore. The first observation with regard to Fig. 5 is that, although the migration of replicators between pores is absolutely

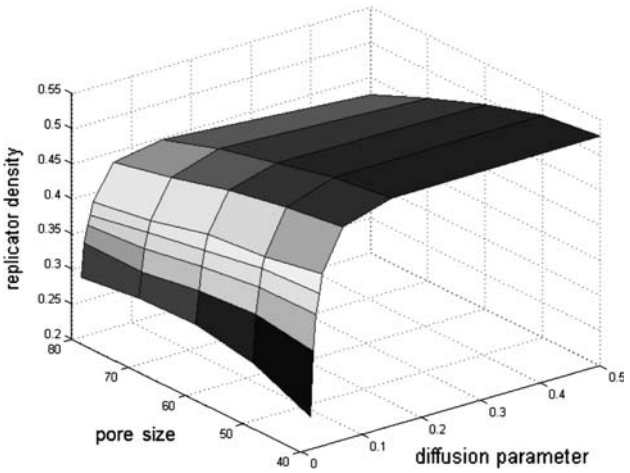

Fig. 5 Effect of migration and pore size on the total replicator density. Fixed parameters: $r = 2$; system size: $I = 5$

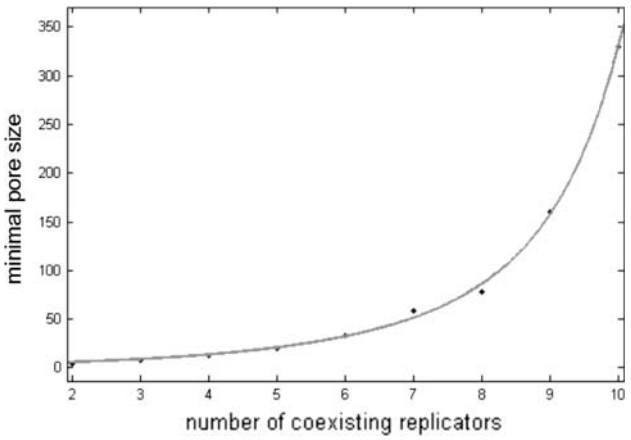

Fig. 6 Relationship between system size I and minimal pore size s necessary for coexistence. The migration parameter was $d = 0.8$

necessary for the system to be viable, it is sufficient to assume a very low replicator mobility ($d = 0.015$) for that, and further increase in the migration parameter above as small a value as $d = 0.1$ makes no significant difference in the performance of the metabolic system.

Changing pore size has a more complicated effect on coexistence, because it depends on the actual mobility of the replicators, but the pore size effect is not very conspicuous either. As Fig. 5 shows, at low migration probabilities (small values of d) an increase in pore size slightly increases the steady state density of the replicator system. This is probably due to the effect of delayed extinction in larger pores (cf. Fig. 3): very low migration mobility often results in local extinction of the replicators in small pores before a viable set of colonizers could be sent out to neighboring empty pores. The risk of local extinction before viable colonization is smaller in larger pores in which local populations persist for a longer time and have higher numbers of potential migrants. This positive effect of pore size vanishes with increasing the mobility of the replicators (larger d), and two other, more pronounced negative effects come into play at the same time. The first one is that larger pores have time to develop a more imbalanced distribution of replicators, because the system goes closer toward the extinction of the least competitive replicator type—i.e., local replicator population dynamics approaches the mean-field case which is known to be doomed to ultimate replicator extinction. Therefore, larger pores maintain less efficient stationary metabolisms than smaller ones, which effect decreases the overall stationary density of the replicators. The other negative effect of larger pore size is the reduction of between-pore variation (Czárán and Szathmáry 2000) which would help the least competitive replicator types to escape extinction, provided that the local populations (i.e., the pore size s) were sufficiently small. Both these negative effects are rather weak within the reasonable range of s, however, so the consequent decrease in stationary replicator densities is quite modest: doubling the pore size from 40 to 80 results in a 20% decrease of replicator density (defined as the fraction of replicators in percentage of the total carrying capacity of the system) at most.

There is an obvious constraint on system size (i.e., the number of different replicator types nr necessary for metabolism to work): the minimal metabolic system must fit into a single pore, that is, $s \geq nr$ is the absolute lower limit of s. In fact, minimal viable pore size appears to increase with system size in an exponential fashion (Fig. 6). The striking fact in this respect is that it is by far easier to maintain a larger system sizes in this model than in the nonporous surface model (Czárán and Szathmáry 2000). We shall detail this result in the "Discussion" section.

Parasite Resistance

Mild and aggressive parasites have different effects on the metabolic system. At reasonably small values of its invasion rate ($r_i \leq 10^{-4}$), the mild type parasite is virtually eliminated (Fig. 7). At higher rates of mild parasite invasion, the metabolic efficiency of the system decreases, simply due to the dilution of cooperators and the resource competitive effect (monomer uptake) of the parasites which are continuously reintroduced into the system by frequent events of invasion (Fig. 8). Yet, metabolism keeps running even at relatively high invasion rates, and the system maintains a steady frequency of mild parasites, which is a very important feature of this model from the viewpoint of its evolvability—we shall return to this point in the "Discussion" section.

Aggressive parasites ($K = 20.0$) can be confined and eliminated from the system under more restrictive conditions: both their invasion and migration rates should be small for the metabolic system to survive their presence (Fig. 9). Invasion rates above $r_i = 10^{-3}$ are lethal (Fig. 8): severe resource competition for monomers and the dilution of cooperators demolish metabolism. High rates of migration ($d \geq 10^{-1}$) have essentially the same effect: infected pores spread many copies of the parasite to many (so far uninfected) pores before their local metabolism breaks down and the lack of monomers kills off the parasite locally. However, migration parameters below $d = 10^{-1}$ are sufficiently low for the containment and elimination of even the aggressive parasite within localized clumps of pores (Fig. 10), which ensures the persistence of the infected metabolic system. Note that below $d = 2 \times 10^{-2}$ the infected system actually benefits from increased migration, because such small mobility is sufficient to help the spread of the weakest cooperator (as explained in the

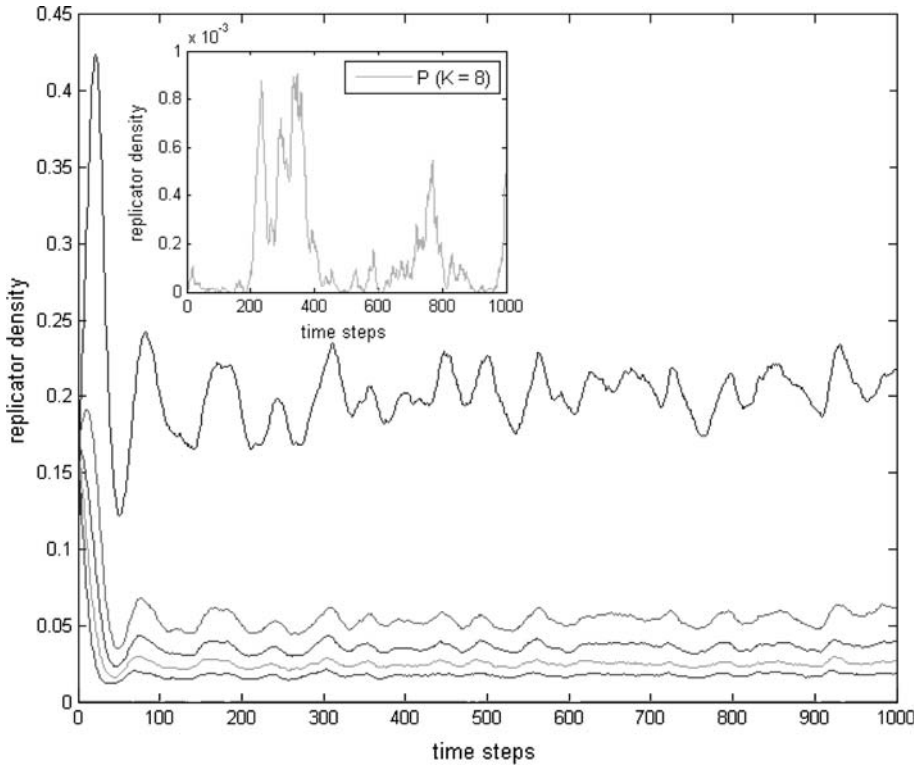

Fig. 7 Population dynamics of five coexisting replicators in the presence of mild parasite populations ($K = 8$). The parameters are the same as in Fig. 3. The inset shows the population dynamics of the parasites

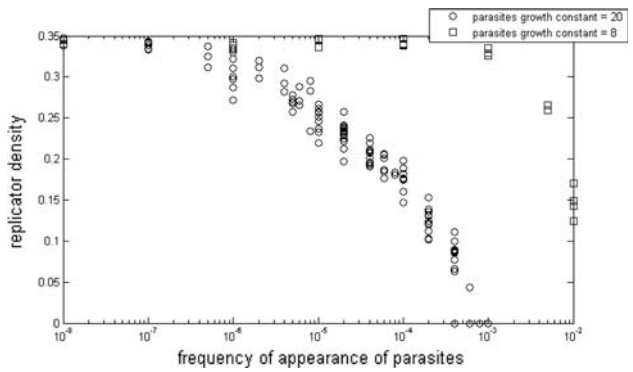

Fig. 8 The effect of mild and aggressive parasites on total replicator density within the metabolic system. Mild: $K_m = 8.00$; aggressive: $K_a = 20.00$. The parameters are the same as in Fig. 3

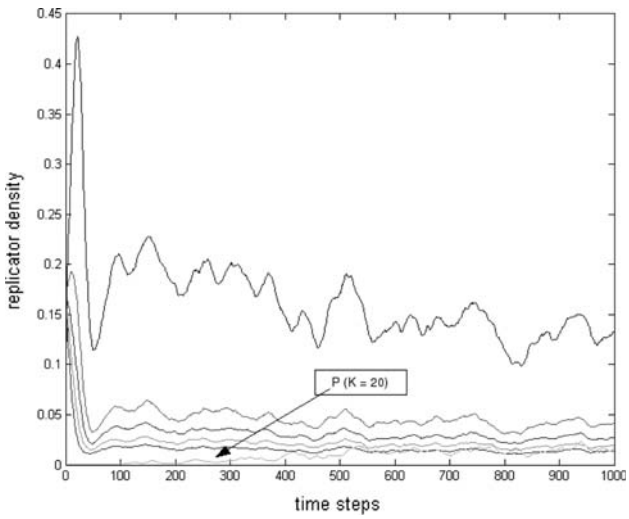

Fig. 9 Population dynamics of five coexisting replicators in the presence of aggressive parasites ($K = 20.0$) shown by the *black arrow*. The parameters are the same as in Fig. 3

previous section) but does not allow the parasite to flush the habitat. The spatiotemporal pattern of a controlled parasite infection of the metabolic system is illustrated in Fig. 11.

Discussion

We have tested the theoretical feasibility of a pre-cellular mineral compartmentalization ("mineral honeycomb") scenario for the evolutionary dynamics of the metabolic replicator system (MRS). It has been demonstrated previously (Czárán and Szathmáry 2000; Könnyü et al. 2008) that surface metabolism can keep the MRS coexistent and protected from harmful parasites due to neighbor-modulated selection within small patches of the habitat. However, the number of different replicators that can be maintained in a persistent metabolic system on a flat mineral surface is rather limited—and the reason for this is exactly the fuzzy nature of group selection in the flat-

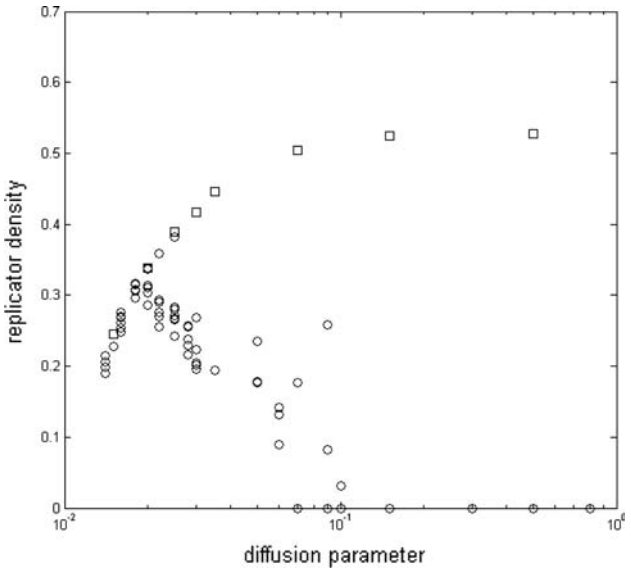

Fig. 10 Effect of the migration parameter d on total replicator abundance in the presence (*red circle*) and absence (*blue square*) of aggressive parasites (growth constant $K = 20.0$). All other parameters as in Fig. 3

surface scenario. A more efficient selection mechanism requires somewhat more distinct entities to select from. Porous minerals like feldspars offer themselves as natural stages for the evolution of replicators in such compartmentalized, distinct groups and thus allow for the coexistence of many more different types of metabolically cooperating replicators than flat mineral surfaces do. The resulting higher diversity of coexistent replicators involves the possibility for the evolution of more efficient metabolisms (i.e., more complex metabolic reaction networks).

It has to be stressed here that the "mineral honeycomb" scenario does not seize the diversity-maintaining potential of the flat-surface scenario (Czárán and Szathmáry 2000) at all: we consider each pore as a distinct, well-stirred reactor in the present model, not using the surface of the inner walls of the pores for spatial selection on the individual level. In this sense, this system represents a worst-case scenario from the viewpoint of replicator persistence. Yet, the flat-surface model could be easily applied to the internal dynamics of each pore, thus neighbors-modulated selection (besides pore-level selection on the replicator

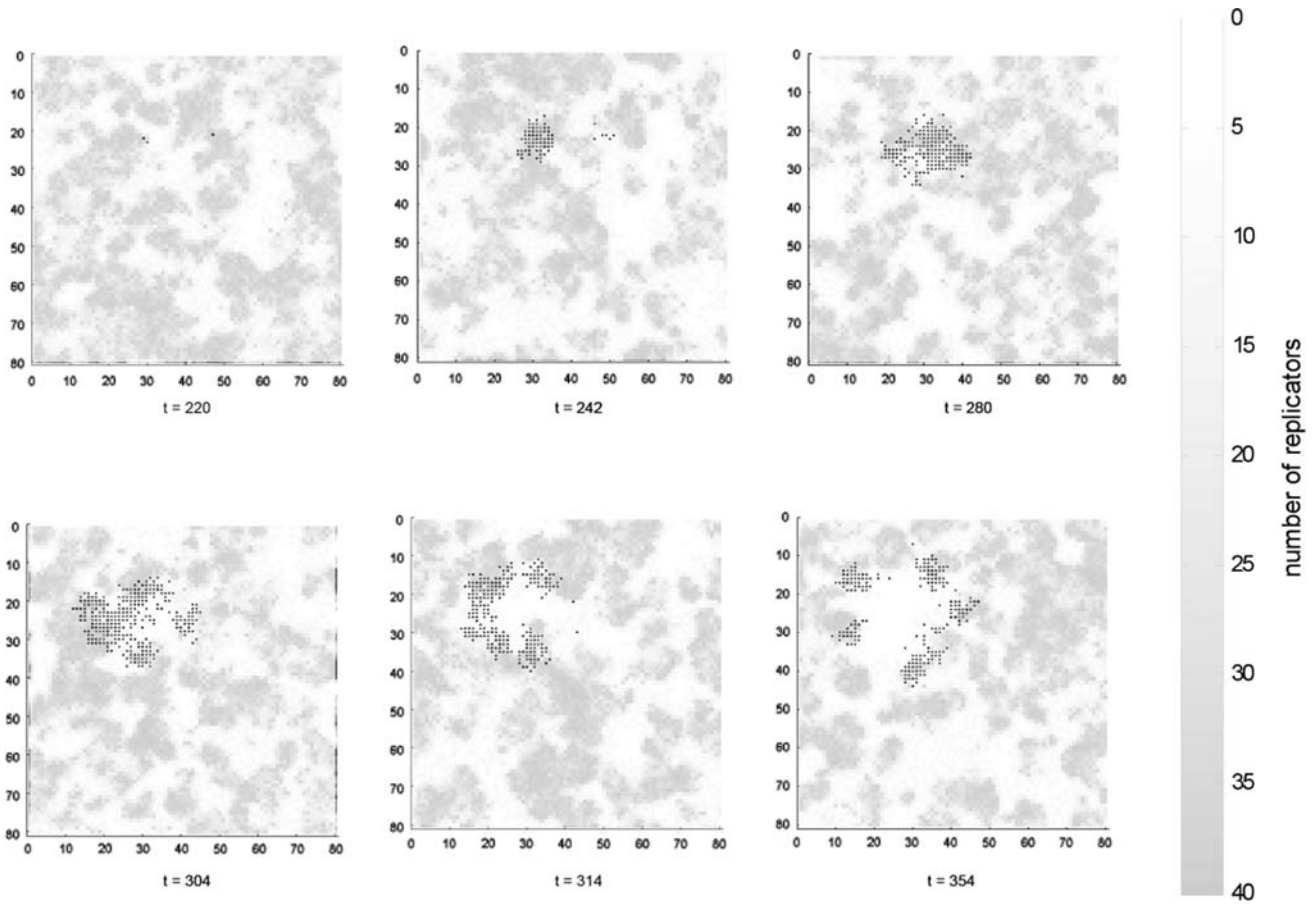

Fig. 11 Spatial representation of the pore system in the presence of aggressive parasites. Each cell in the grid represents a pore; replicator abundances are shown in the *light grayscale*. The presence of at least one parasite in the pore is marked by a *black dot*

populations of the pores) and increasing the propensity for persistence and gradual metabolic perfection of the resulting "mineral honeycomb-surface" system. The corresponding two-level selection model remains to be built and studied in the future.

Two important points are due to with regard to the appearance and the evolution of parasitic replicators in the pore model. First, we have seen (cf. Fig. 7) that very aggressive parasites can kill the pore system if they have a high invasion rate. The question here is whether or not it is realistic to assume a sufficiently high rate of aggressive parasite invasion that affects the system seriously. Invasion might be external (parasites arriving from outside the honeycomb) or internal: parasites produced as mutants of the resident replicators within the pore system. External invasion at sufficient numbers requires the presence of a nearby source of dangerous parasites, but such a source is unlikely to exist for long: it commits suicide soon and stops emitting the pests. The only real danger is the fast internal production of aggressive mutants, which cannot be excluded (broken cooperators—i.e., deletion mutants—might act as such parasites, for example). However, such deletion mutations might not appear at or above the critical rate. It remains to be seen whether the introduction of neighbor-modulated fitness dynamics within the pores keeps such parasites at bay, as it was demonstrated for surface metabolism (Czárán and Szathmáry 2000).

The other point to make on parasite evolution is related to evolvability that we have already stressed (and partly studied) in three other papers (Czárán and Szathmáry 2000; Könnyü et al. 2008; Scheuring et al. 2003). We consider this aspect the most exciting one in the metabolic system: its capability to co-opt additional types of cooperating replicators via mutational changes in mild parasites, while keeping harmful mutants in check. Since parasites are always present and do not do real harm to the metabolic system other than just tapping it for monomers to be used in their own replication, there are no serious constraints on their primary structure (monomer sequence): they can freely mutate. Harmful mutants are eliminated by trait group selection (cf. Fig. 7), but some of the mutants might occasionally offer some beneficial "service" to the metabolic system that makes it more efficient in some way. In what ways converted parasites might benefit the system is rather straightforward: by making metabolism work better (i.e., by opening new reaction routes that help monomer production—Fig. 12a), by obtaining some replicase activity somewhat better than offered by the mineral substrate (Fig. 12b), or by producing membrane components that ultimately leads to the containment of the system in membrane vesicles (Fig. 12c). Obviously, all such mutants are of high benefit for the metabolic system: better metabolism delivers more monomers; a replicase helps replication in terms of both speed and accuracy; and a membrane envelope allows the invasion of new habitats and more efficient selection regimes for the system in a proto-cellular structure. All these benefits might be achieved through gradual evolution, starting from mild parasitic replicators adopting a minimum of the corresponding enzymatic function and then improving it with small mutational steps. We have actually demonstrated that this process can work, in a detailed simulation model for the case of the replicase function in Könnyü et al. (2008).

The combination of such beneficial mutations can be brought together within the same "lineage" of the metabolic system by a series of mutation-selection processes, but it can also be the result of recombination between similar, but not identical, evolved systems. In any case, the resulting proto-cellular "organism" (Fig. 12d) (an incarnation of Gánti's catalytic chemoton—(Gánti 2003) might become independent of the mineral substrate as soon as the cooperating replicators provide better enzymatic functions and (in the form of dividing membrane vesicles) a better stage for group selection than the flat mineral surface or the mineral honeycomb can offer. The feasibility of this "abstriction from the surface" (Wächtershäuser 1992) or

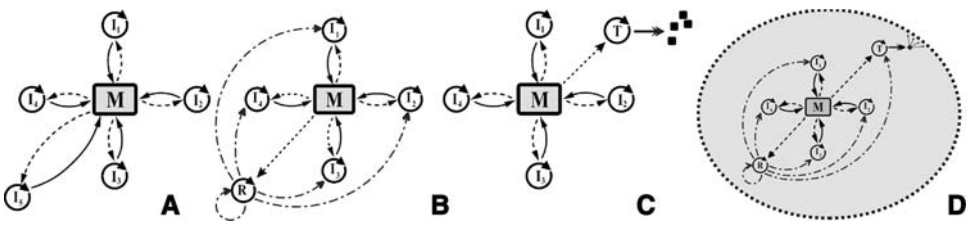

Fig. 12 Conversion of persistent parasites into cooperators: the evolution of new functions in the metabolic system. **a** Parasite mutates into a new metabolic enzyme I_5; **b** Parasite R obtains replicase activity (slightly better than that of the mineral surface); **c** Parasite T evolves into an enzyme that produces membrane units (amphipatic molecules). M represents the metabolic reaction network providing monomers for replication (*dashed arrows*), I_i are replicators with metabolic enzyme activities (*full arrows*). The replicase replicator (R) aspecifically helps the replication of all types of replicators (*dashed-dotted arrows*); the membrane-producing replicator T converts metabolic wastes into membrane units (*double-headed arrow*); **d** The complete proto-cell capable of life independent of the mineral honeycomb

"prebiotic takeoff" scenario of metabolic system evolution remains yet to be studied. Our main result in this paper is the support for the importance in prebiotic evolution of inorganic compartments (Koonin and Martin 2005).

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