Competitive Exclusion among Self-Replicating Molecules Curtails the Tendency of Chemistry to Diversify

Marcel J. Eleveld, Yannick Geiger†, Juntian Wu†, Armin Kiani, Gaël Schaeffer and Sijbren Otto

Centre for Systems Chemistry, Stratingh Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Abstract

The transition of chemistry into biology is poorly understood. One of the key questions in this transition is how the inherently divergent nature of chemical reactions can be curtailed, allowing product mixtures to become enriched in only a limited subset of all possible reaction products. Another seemingly unrelated question is whether Darwinian principles from biology extend to chemistry. Addressing both questions simultaneously, we now show that the evolutionary principle of competitive exclusion, which states that a single niche can only be stably occupied by one species, also applies to self-replicating chemical systems, and that this principle diminishes the tendency of chemistry to diversify. Specifically, we report two systems in which three different self-replicator quasi-species emerge in a largely stochastic fashion from a mixture of two building blocks (resources). To enable their evolution, these replicator mixtures were subjected to an out-of-equilibrium replication-destruction regime, implemented by serial transfer. Out of the many different products initially produced, competitive exclusion leads to the selection of only a single quasi-species when all replicators rely to the same extent on both resources. When, on the other hand, one of the quasi-species preferentially uses one resource and another quasi-species specializes in the other (resource partitioning), these replicator quasi-species effectively occupy different niches and were found to coexist in an evolutionary stable steady state. The ability to escape from competitive exclusion through resource partitioning is important for future efforts on addressing a major evolutionary challenge on the path to life's emergence: Eigen's paradox, which requires evolutionary stable communities of co-existing replicators with specific community dynamics.

Introduction

How chemistry can transition into biology is one of the grand challenges of modern science, which has raised several fundamental questions: Do the laws of biology extend to abiotic chemical systems? Do Darwinian principles play a role in turning chemistry into biology? How can the inherent tendency for reactive chemical mixtures to become more diverse be reversed, allowing mixtures to become enriched in specific molecules? Research on synthetic chemical systems that start to show life-like properties can provide answers to these questions. Self-replication is one such life-like property, and self-replicating molecules are likely to have an important role in the transition of chemistry into biology.[1,2] In principle, Darwinian evolution may be expected to act on abiotic self-replicating systems. However, in practice, demonstrating evolutionary phenomena in these systems has proven difficult. The first hurdle that has to be overcome is achieving exponential self-replication. The vast majority of synthetic self-replicating systems that have been developed to date show only sub-exponential growth, which, under most experimental conditions, will lead to co-existence of competing replicators. Yet, Darwinian evolution involves survival of the fittest and extinction of the weaker competitors. Such dynamics effectively needs exponential replicators,[3,4] of which there are only a few.[5–8] A second requirement is that self-replication occurs in a regime that allows for evolution, where replication is accompanied by replicator destruction (death; implementable by serial transfer,[9–12] flow setups,[13] or by chemical means[14]). The majority of experiments on self-replication have been run in closed systems, lacking a process equivalent to death. In such systems the distribution of products is typically governed by thermodynamics, while under out-of-equilibrium conditions relevant for evolution, dynamic kinetic stability (persistent state of an open chemical system subject to separate formation and destruction processes)[15,16] governs the distribution of replicators. However, recently, self-replication is starting to be investigated away from equilibrium.[10,13,14]

Now that exponential self-replicators have become available and the methodology of replication in an out-of-equilibrium replication-destruction regime has been established, we are in a position to start probing the degree to which Darwinian principles extend into chemistry. Among these, the competitive exclusion principle is of particular relevance. This principle states that a single niche can only be stably occupied by one species.[17,18] In ecology, when two species rely on the same food source (niche) the one that has a slight advantage will lead to the extinction of the other (or forces it to move to a different habitat).[19,20] Two species can, on the other hand, stably coexist when they specialize in different available resources. This resource partitioning (or niche differentiation) provides the basis for different plant species to coexist[21] and is essential for maintaining species diversity.[22] Theoretical work suggests that resource partitioning is also essential for coexistence of prebiotic (self-)replicating templates,[23] but this has not been demonstrated experimentally yet.

The competitive exclusion principle may fulfil an important role in taming the natural tendency for chemistry to diversify due the high cross-reactivity between molecules, leading to a rapid increase in the number of different molecules. Competitive exclusion can curtail diversity, which is probably necessary for chemistry to transition into biology. However, at the same time, a limited degree of diversity is needed. Diversity of co-existing self-replicators provides a solution to another hurdle in early evolution: Eigen's paradox,[24] which states that self-replicators require a lot of information to be able to replicate accurately, but in order to obtain a self-replicator that contains a lot of information, an accurate replication process is required. The different theoretical solutions to this paradox[25–28] all involve dividing the information over several separate co-existing self-replicators which then cooperate such that selection acts on the community.[1] Thus, at some point in the transition of chemistry into biology, replicator communities are likely required. Research on biology suggests that resource partitioning may provide a means to achieve co-existence of self-replicators allowing evolutionary stable communities to form.

We reasoned that the questions whether systems of synthetic self-replicating molecules could exhibit competitive exclusion and thereby limit chemical diversity, and whether the exclusion principle could be lifted by resource partitioning, could be addressed using the systems of self-assembly based self-replicators we developed previously.[2] In these systems pseudo-peptide building blocks (Figure 1a) can spontaneously form self-replicators in an agitated mixture.[5,29] When dithiol-bearing building blocks, appended with a short peptide, are dissolved in an aqueous buffer, slow oxidation by oxygen from the air results in the formation of a mixture of macrocycles that constantly exchange building blocks through thiol-disulfide exchange (Figure 1b). If a macrocycle of a specific size is able to self-assemble into fibres, through a combination of β-sheet formation and π-stacking of the aromatic core unit,[30] the composition of the mixture will shift to produce more of the self-assembling macrocycle, resulting in self-replication. Mechanical agitation of the sample causes fragmentation of fibres with a sufficient length, increasing the number of growing fibre ends, pushing the replication process into an exponential regime (Figure 1c).[8]

We now show that allowing two different dithiol building blocks to oligomerise under kinetic control results in a diverse set of products. Diversity is curtailed drastically after a set of replicators quasi-species (winning subsets of replicators in a mutation-selection balance, that comprises a cloud of related structures with high mutation rates[24,31]) emerged and was allowed to evolve. Competition of these different quasi-species was found to obey the competitive exclusion principle. When the quasi-species rely to the same extent on both resources, subjecting them to a replication/destruction regime (implemented by serial transfer) led to the persistence of only one. We also demonstrate in another system that coexistence of a limited number of quasi-species becomes possible when the replicators occupy different niches, which can be achieved by resource partitioning (preferential incorporation of one of the building blocks over the other by one quasi-species, while the other has opposite preferences).

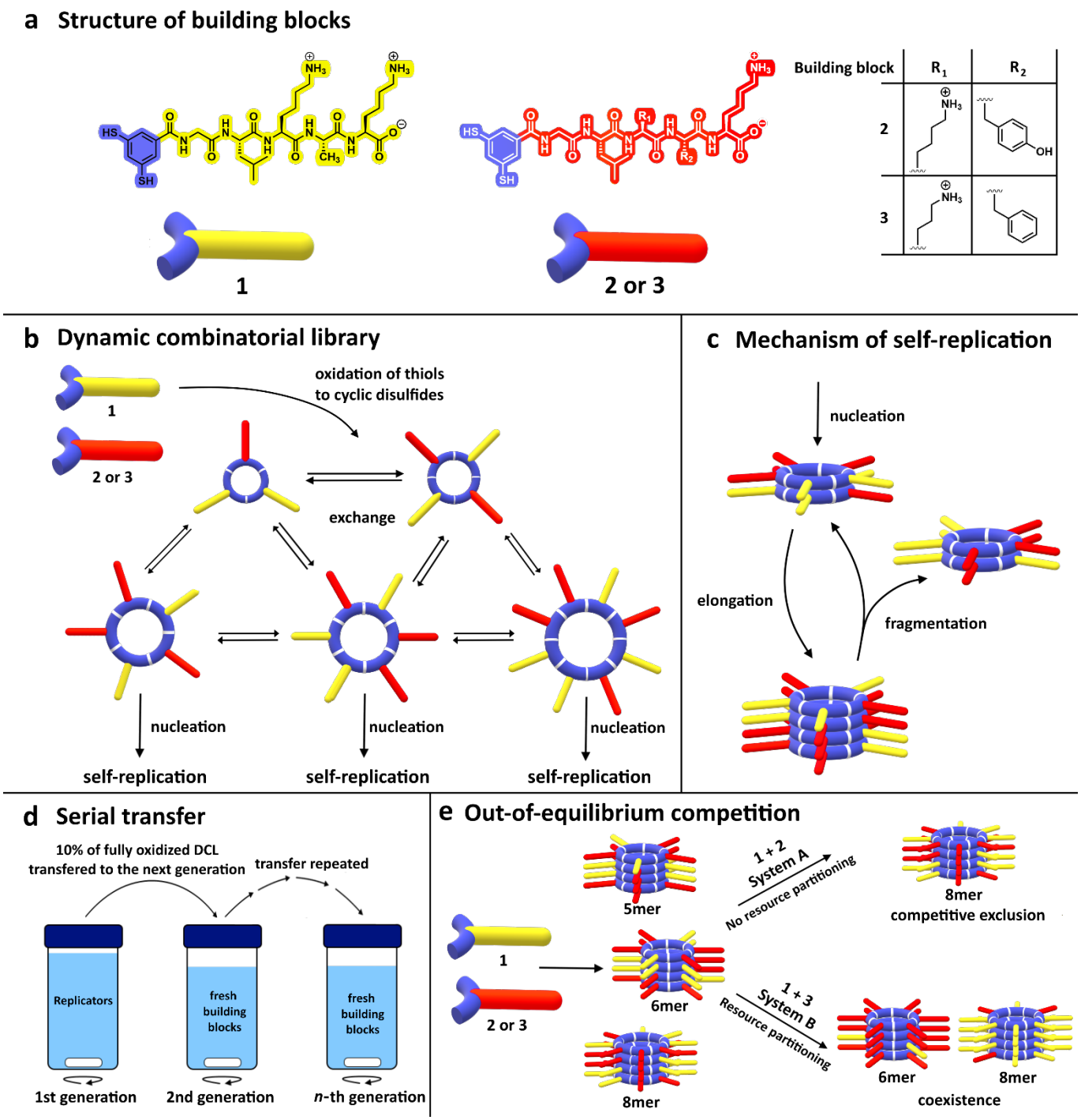

*Figure 1. (a) Structures and graphical representation of building blocks. System A consists of equimolar amounts of **1** and **2**, while system B consists of equimolar amounts of **1** and **3**. (b) When thiols are slowly oxidized to disulfides by oxygen from the air, a DCL of macrocycles is formed that constantly exchange building blocks through thiol-disulfide exchange. Separate nucleation events can cause macrocycles of specific sizes (pentamers, hexamers or octamers) to self-assemble into fibres (shown for the pentamer in panel c), shifting the equilibrium of the DCL to produce more of the very macrocycle that self-assembles. (c) Fibers of a sufficient length are fragmented by mechanical agitation through stirring, resulting in exponential growth of the fibres. The elongation/fragmentation mechanism is only shown for the pentamers, but the other self-replicators also replicate using this mechanism. (d) Procedure of serial transfer. After oxidation is essentially completed 10% of the DCL is transferred to a vial containing fresh building blocks (1.0 mM total concentration; 25 mM B_2O_3-buffer; pH 8.18) that had been rapidly oxidized to 50% disulfides using sodium perborate ($NaBO_3$; 40 mM) prior to transfer. The new DCL was stirred (1200 rpm) for 4 days at 45 °C to allow essentially complete conversion of all thiols to disulfides. Transfer to a fresh solution was performed several times generating a total of n generations. (e) In the absence of resource partitioning (system A) the fittest replicator displaces all others when they have to compete for their common resources in an out-of-equilibrium regime, implemented by serial transfer. Resource partitioning (occurring in system B) allows different replicators to coexist in an out-of-equilibrium regime.*

Results and discussion

We focused our studies on two binary combinations of building blocks: system A, made from dithiol building blocks **1** and **2**, and system B, obtained upon mixing building blocks **1** and **3** (Figure 1a). These building block combinations were selected following a large survey of dynamic combinatorial libraries (DCLs) prepared from binary mixtures of building blocks, where these combinations stood out by their ability to give rise to several different-sized replicators. The inherent tendency of chemistry to diversify was evident when equimolar amounts of building block **1** with either **2** or **3** were mixed and rapidly oxidized, using perborate as an oxidant, to obtain kinetically controlled reaction products. Under these conditions the oxidation of thiols to disulfides is faster than disulfide exchange. Ultra-performance liquid chromatography – mass spectrometry (UPLC-MS) analysis showed that complex mixtures of oligomeric disulfide-linked macrocycles with diverse compositions and ring sizes, ranging from 3 to 18 monomer units, were produced (top traces in Figure 2a and b; see SI sections III.1 and III.5.1 for a detailed chromatographic analysis of System A).

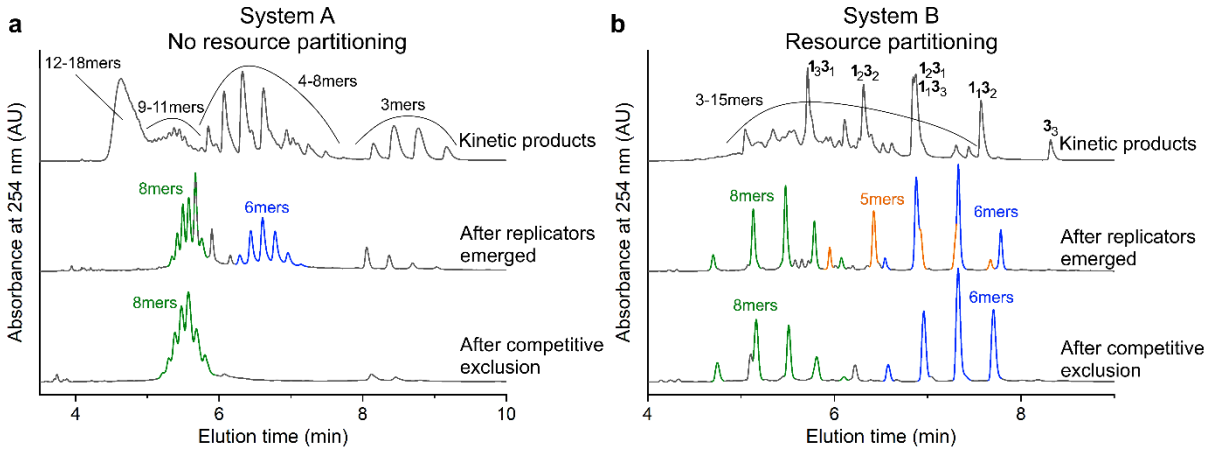

*Figure 2. Normalized UPLC-chromatograms (recorded at 254 nm) showing different product distributions obtained upon mixing equimolar amounts of (a) **1** and **2** (system A) or (b) **1** and **3** (system B). Rapid oxidation leads to a large variety of products, with 3-18 membered macrocycles being detected (top traces). A reduced variety of products was obtained when self-replicators were allowed to emerge under conditions that disulfide exchange takes place (middle traces). In these systems replicator quasi-species emerge with three different ring sizes: 5mer (orange), 6mer (blue) and 8mer (green). The variety of products was reduced further when the replicator quasi-species were allowed to evolve while having to compete for their common building blocks, leading to competitive exclusion (bottom traces). Competitive exclusion leads to the persistence of only a single replicator quasi-species, in case all quasi-species rely to the same extent on the available resources (panel a) or the co-existence of two quasi-species, when these occupy different niches through resource partitioning (panel b).*

System A: competitive exclusion. Recently, we reported that two different populations of replicators (hexamers and octamers) can emerge from system A.[32] Both populations of self-replicators are initially formed by stochastic nucleation events. In an equimolar mixture of **1** and **2** both replicators were found to have similar chances of nucleation, similar growth kinetics and very little (or no) cross-catalysis towards each other. These experiments were conducted in closed systems, where replicator destruction was not actively promoted and no evolution could take place.

Because both populations of replicators (hexamers and octamers) can be produced from the same building blocks this is an ideal system to explore the effect of evolution on the replicator composition. Analysis by UPLC (Figure 3a) showed that the hexamer and octamer replicators incorporate building blocks **1** and **2** in similar and close to statistical amounts (Figure 3c+e), which implies that they occupy the same niche. If the competitive exclusion principle holds here, then only one of the replicators will be able to survive at the expense of the other once the replicator system is allowed to evolve.

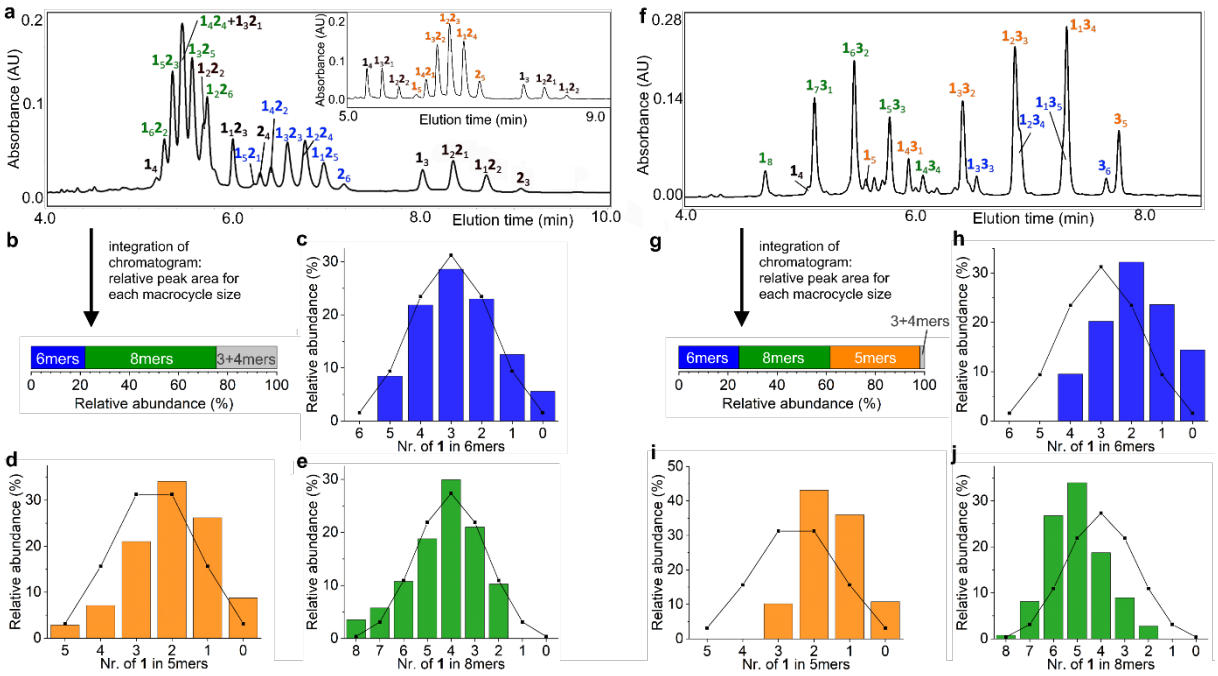

*Figure 3. (a) UPLC-chromatogram (recorded at 254 nm) of a dynamic combinatorial library (DCL) made from equimolar amounts of **1** and **2** after 4 days. In this DCL both 6mer and 8mer replicators can be observed. The inset shows a chromatogram of a DCL where 5mer replicators are dominant. (b) By integration of the peaks in the chromatogram shown in panel a the relative abundances of the various replicators can be determined. (c-e) Relative abundances of the various macrocycle compositions (expressed in the number of units of **1** incorporated into the macrocycle) obtained for 6mer, 5mer and 8mer replicators, respectively. (f) UPLC-chromatogram (recorded at 254 nm) of a DCL made from equimolar amounts of **1** and **3** after 4 days. In this DCL 5mer, 6mer and 8mer replicators are present. (g) By integration of the peaks in the chromatogram shown in panel f the relative abundances of the various replicators can be determined. (h-j) Relative abundances of the various macrocycle compositions (expressed in the amount of **1** incorporated into the macrocycle) obtained for 6mer, 5mer and 8mer-replicators, respectively. The black lines in panels c-e and h-j show, as a reference, a statistical (or random) incorporation of both building blocks in each replicator. The relative peak areas can be used as a measure of the composition of the DCL, because the total peak area in the UPLC analysis remains constant throughout the experiment (Figure S24).*

As a starting point for evolutionary experiments, we prepared ten DCLs by dissolving **1** and **2** into an aqueous buffer (1.0 mM total building block concentration in borate buffer (50 mM in B atoms, pH 8.2)), which were rapidly oxidized to 50% disulfides by adding a sodium perborate solution ($NaBO_3$, 100 mM). The DCLs were deliberately prepared to yield different ratios between the hexamer and octamer replicators. This was done by seeding with 10 mol% of pre-formed replicators or by varying the initial ratio between **1** and **2** (see Table S1). Changing the ratio between **1** and **2** will shift the replicator distribution towards hexamers when there is more than 50 mol% **2** or octamers when there is more than 50 mol% of **1**.[32]

Having different starting compositions allows probing the influence (if any) of this parameter on the evolutionary fate of the systems. After stirring the 10 DCLs (1200 rpm) at 45 °C for 4 days essentially all thiols were converted to disulfides and strongly varying library compositions were obtained with relative abundances ranging from 3% to 69% for hexamers and from 6% to 74% for octamers. Subsequently these DCLs were used to seed the second generation of replicators by serial transfer (Figure 1d). Ten mol% of the fully oxidized DCLs were transferred to fresh solutions of equimolar amounts of **1** and **2**, oxidized to 50% disulfides followed by stirring at 45 °C for 4 days. This serial transfer process was performed four times, producing five generations of replicators. Note that, in the limit of many transfers, the dynamics of serial dilution resemble that of a continuously stirred tank reactor, in which building blocks flow into the reactor and the produced replicators flow out of the reactor with the same flow rate.[33]

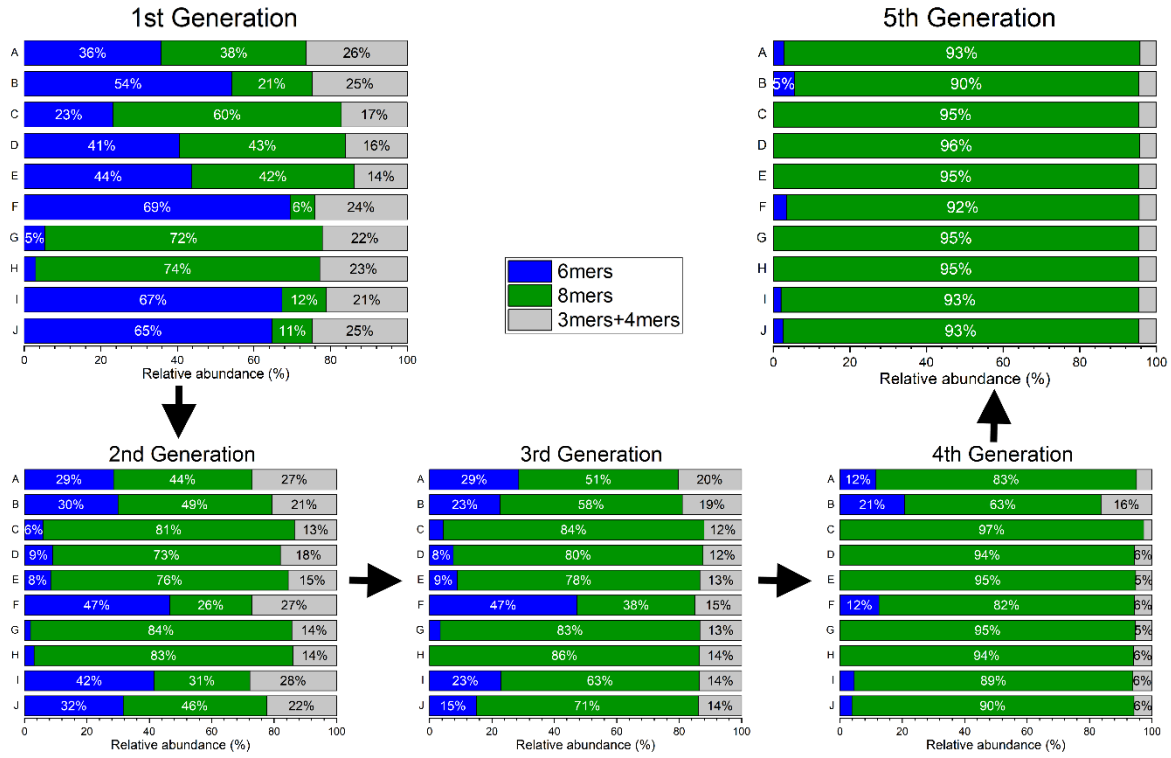

*Figure 4. Evolution of replicator populations in system A made from equimolar amounts of **1** and **2** (1.0 mM total concentration in 25 mM B_2O_3-buffer, pH 8.18) in a replication-destruction regime, implemented by serial transfer. The first generation was prepared as shown in Table S1, stirred for 4 days (1200 rpm; 45 °C) and analysed by UPLC-MS. A small aliquot (10%) of each DCL was transferred to a fresh solution of **1** and **2** (oxidized to 50% disulfides) that was then also stirred for 4 days (1200 rpm; 45 °C) yielding a second generation. This process was repeated three more times to yield a total of five generations.*

With each generation the amount of octamer replicators in each DCL increased at the expense of the hexamer replicators. After four serial transfers (fifth generation) almost no hexamers could be detected anymore (Figure 4). Apparently the octamers constitute the quasi-species with the highest dynamic kinetic stability in this out-of-equilibrium regime, causing the demise of the hexamers.

It is also possible to produce a population of pentamer replicators in this system by seeding with $\mathbf{2}_5$ (Figure S1a).[34] Two DCLs were prepared in this way, which, after four days, contained pentamers in relative abundances of 71% and 74%. These pentamers, like the hexamers and octamers, incorporate building blocks **1** and **2** in similar amounts (Figure 3d). Also small amounts of octamers (<5%) could be detected and some residual small macrocycles (3mers and 4mers). These DCLs were also used as starting points for serial transfer experiments, performed as described before. After nine serial transfers (tenth generation) almost no pentamers could be detected and all DCLs were dominated by octamers (Figure 5; note that the layout differs from that of Figure 4 and 6). These data illustrate that, even when the difference in dynamic kinetic stability of two competing replicator quasi-species populations is relatively small and the bias is initially towards the less stable quasi-species, the one with the highest dynamic kinetic stability will ultimately displace competitors that occupy the same niche.

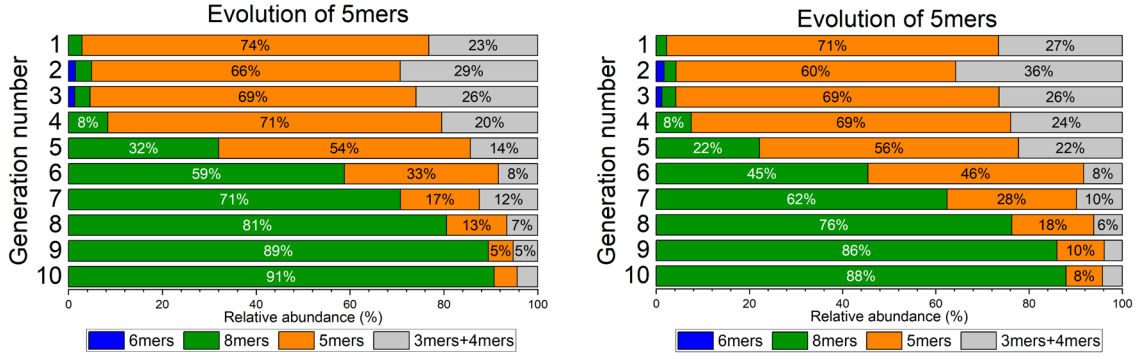

*Figure 5. Evolution of two pentamer replicator populations in system A made from equimolar amounts of **1** and **2** (1.0 mM total concentration in borate buffer, 50 mM in B atoms, pH 8.18) in a replication-destruction regime, implemented by serial transfer. The first generation was*

*prepared as shown in Table S2, stirred for 4 days (1200 rpm; 45 °C) and analysed by UPLC-MS. A small aliquot (10%) of each DCL was transferred to a fresh solution of **1** and **2** (oxidized to 50% disulfides) that was stirred for 4 days (1200 rpm; 45 °C) yielding a second generation. This process was repeated another eight times to yield a total of ten generations.*

System B: coexistence through resource partitioning. The results above show that the competitive exclusion principle from evolutionary biology also extends to replicator chemistry. The competitive exclusion principle also implies that two populations (of replicators) can only stably coexist in a replication-destruction regime when they occupy different niches. We therefore also investigated System B, consisting of **1** and **3**. This system was part of a large screening of equimolar mixtures of different building blocks that form self-replicators and piqued interest because in batch conditions three different replicators (pentamers, hexamers and octamers) that have different building block preferences could be identified in a single DCL (Figure 2b and 3f; see SI sections III.1 and III.5.2 for a detailed chromatographic analysis). Seeding experiments confirmed that the three species are indeed replicators (Figures S1b and S2; cf. Figure S3 for the kinetic traces of spontaneous octa- and hexamer emergence from **1**+**3** in a 50:50-ratio). Thioflavin T assays (SI section III.2) and circular dichroism data (SI section III.3) were in line with previous observations on related replicators[5,29,32] suggesting that also the replicators in system B assemble through β-sheet formation. Transmission electron microscopy (TEM) analysis revealed fibrous assemblies, similar to those observed previously in related replicators (SI section III.4).[5,29,32] Similar to System A,[32] also system B shows stochastic effects in the nucleation of the hexamer and octamer replicators that are largest near the phase boundary (Figure S4).

In this system the competing replicators have different building block preferences: octamers are enriched in building block **1** and hexamers and pentamers preferentially incorporate **3** (Figure 3h-j). As is the case for System A, different replicator distributions can be obtained by varying the ratio between **1** and **3**, where an increased amount of **1** promotes octamer formation and an increased amount of **3** benefits hexamer formation (Figure S4). In System B there is also a possibility for small amounts of pentamers to emerge. These pentamers are, just like the hexamers, rich in **3**. The important difference between Systems A and B is that in System B the replicators do not incorporate both available building blocks in a similar proportion. The octamers are enriched in **1**, while the hexamers and pentamers are enriched in **3**. This effectively means that the octamers occupy a different niche compared to the hexamers and pentamers, because they rely to a different extent on the different available resources. We expected that, when DCLs containing replicators made from **1** and **3** are allowed to evolve in a formation-destruction regime, it should be possible for two replicator populations to stably coexist, where one is rich in **1** and the other in **3**.

We prepared ten DCLs by mixing **1** and **3**, in the same way as described above for system A. As for system A DCLs were prepared containing different ratios between the different replicators by varying the initial ratio between **1** and **3** (only for the first generation samples; Table S3) for nine DCLs. The tenth DCL was cross-seeded with 10 mol% of preformed pentamers consisting of **1** and **2** in order to obtain more pentamers.

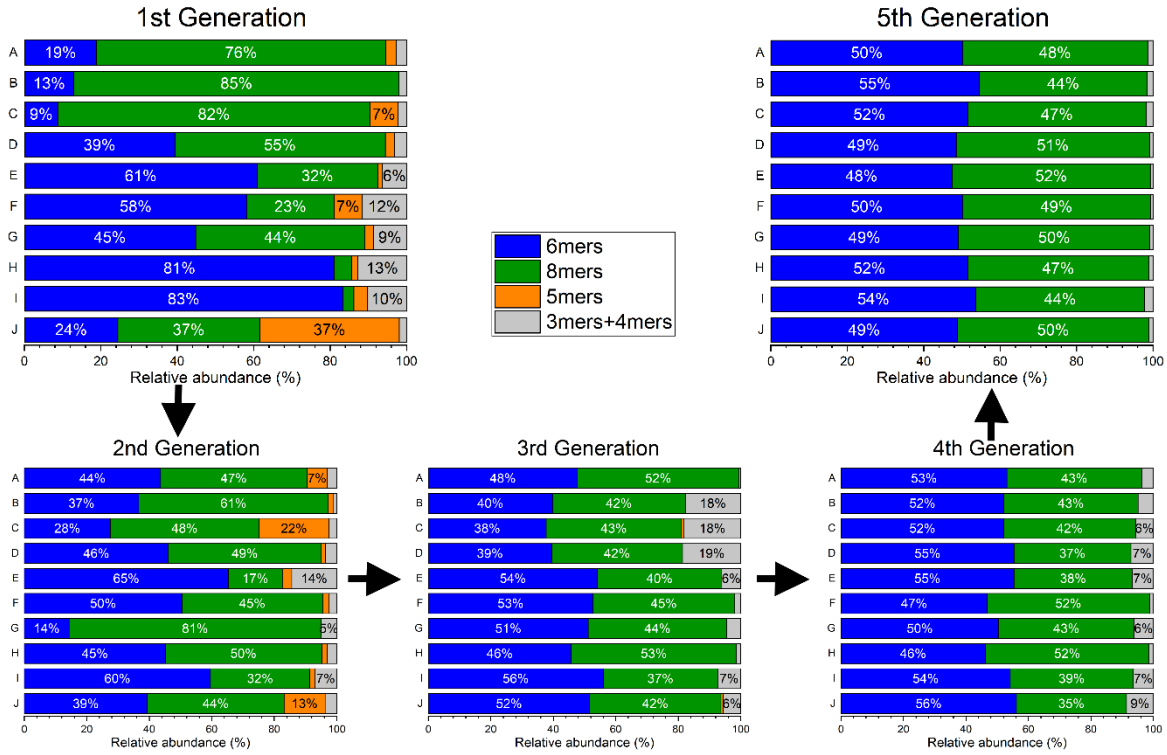

*Figure 6. Evolution of replicator populations in system B made from **1** and **3** (1.0 mM total concentration in 25 mM B_2O_3-buffer, pH 8.18) in a replication-destruction regime, implemented by serial transfer. The first generation was prepared as shown in Table S3, stirred for 4 days (1200 rpm; 45 °C) and analysed by UPLC-MS. A small aliquot (10%) of each DCL was transferred to a fresh solution of **1** and **2** (oxidized to 50% disulfides) that was stirred for 4 days (1200 rpm; 45 °C) yielding a second generation. This process was repeated another three times to yield a total of five generations.*

After stirring the DCLs (1200 rpm) at 45 °C for 4 days the conversion of thiols into disulfides was essentially complete and large variations in library compositions were obtained with relative abundances of the different replicator quasi-species ranging from 9% to 83% for hexamers, from 3% to 85% for octamers and from 0% to 37% for pentamers. These first generation DCLs were used as the starting point for four successive serial transfers conducted in the same way as described above for system A. With each generation the pentamer population decreased in abundance until it could no longer be detected (Figure 6). As the pentamer and hexamer populations occupy the same niche (they both preferentially incorporate **3**; Figure 3h+i), this result is in agreement with competitive exclusion of the population with the lowest dynamic kinetic stability. With each generation the octamer and hexamer populations converged towards a state where both quasi-species have a relative abundance of around 50%, suggesting that they have comparable dynamic kinetic stability. Here it is possible for two quasi-species to stably coexist next to each other because the hexamers and octamers occupy different niches: the hexamers preferentially incorporate **3**, while the octamers preferentially incorporate **1**. Similar to a dual-point entry experiment to identify the thermodynamic product, here various different starting compositions all converge to the same replicator distribution (at steady state at the level of the serial transfer experiments), indicating that this distribution is evolutionary stable under the conditions of these experiments.

Not surprisingly, the outcome of these selection experiments depends on the selection regime. Upon serial transfer, selection is primarily based on replication kinetics, as the rate of "dying" (i.e. not being transferred to the next vial) should be identical for all replicators. We briefly probed the behaviour of systems A and B under a different selection regime, where disulfide bonds were continuously broken (by continuously supplying a reductant) and formed (by simultaneous supply of an oxidant), using a previously established protocol ("redox infusion").[14] Here "death" is no longer indiscriminate, as the rate of reduction tends to be different for different replicators. Indeed, under these conditions system B no longer exhibits coexistence of octamer and hexamer, but yielded mostly hexamer alone (or together with pentamer, when this replicator was there from the start; see Figure S5). We attribute this behaviour to the octamer being reduced more rapidly than the competing replicators (Figure S6), diminishing its relative dynamic kinetic stability. System A proved to be more difficult to study under these oxidation/reduction conditions: only one out of six attempts gave useful results (Figure S7), while others showed rapid loss of total UPLC peak area, discouraging further investigation.

The results obtained with systems B and A show that the substantial chemical diversity produced upon kinetically controlled oligomerization of the two-building-block systems (Figure 2a and b, top traces) is drastically reduced upon the emergence of self-replicators (Figure 2, middle traces) and further curtailed through competitive exclusion that takes place during the evolution of these systems.

Mechanistic insights. System A shows competitive exclusion while system B shows coexistence, even though the building blocks from which the two systems are made only exhibit minor differences in structure, raising the question what causes the marked difference in behaviour. Earlier studies showed that unassembled material accumulates and diffuses on the fibre surface forming a reservoir from which replicators "feed".[35] We now found that differences in binding selectivity of replicator precursors to the side of the replicator fibres is partially responsible for the differences in behaviour of the two systems. We probed the amount and composition of the fibre-bound precursor material by suspending pre-formed fibres of hexamer or octamer in a solution containing their respective precursors, followed by centrifugation. The fibres were found to sediment along with the bound material (control experiments without fibres gave negligible sedimentation). In both systems A and B, hexamer fibres bind more material than the corresponding octamers (Figure 7a,d), especially trimers (Figure 7b,e). While in system A the **1**/**2** ratio in bound trimers and tetramers is essentially identical for hexamer and octamer replicator (Figure 7c), significant differences between the two competing replicators are observed in System B (Figure 7f). Here, $\mathbf{3}_6$ fibres show a strong preference for **3**-rich trimers (even more for **3**-rich tetramers, cf. Figure S8), while $\mathbf{1}_8$ fibres accumulate more **1**-rich macrocycles, as compared to the building-block composition of the precursor solution. This observation is consistent with octamers growing mostly on **1** and hexamers mostly on **3** and suggests that the observed precursor partitioning happens already at the level binding of precursors to the fibres, presumably biasing what material will be incorporated during the replication process.

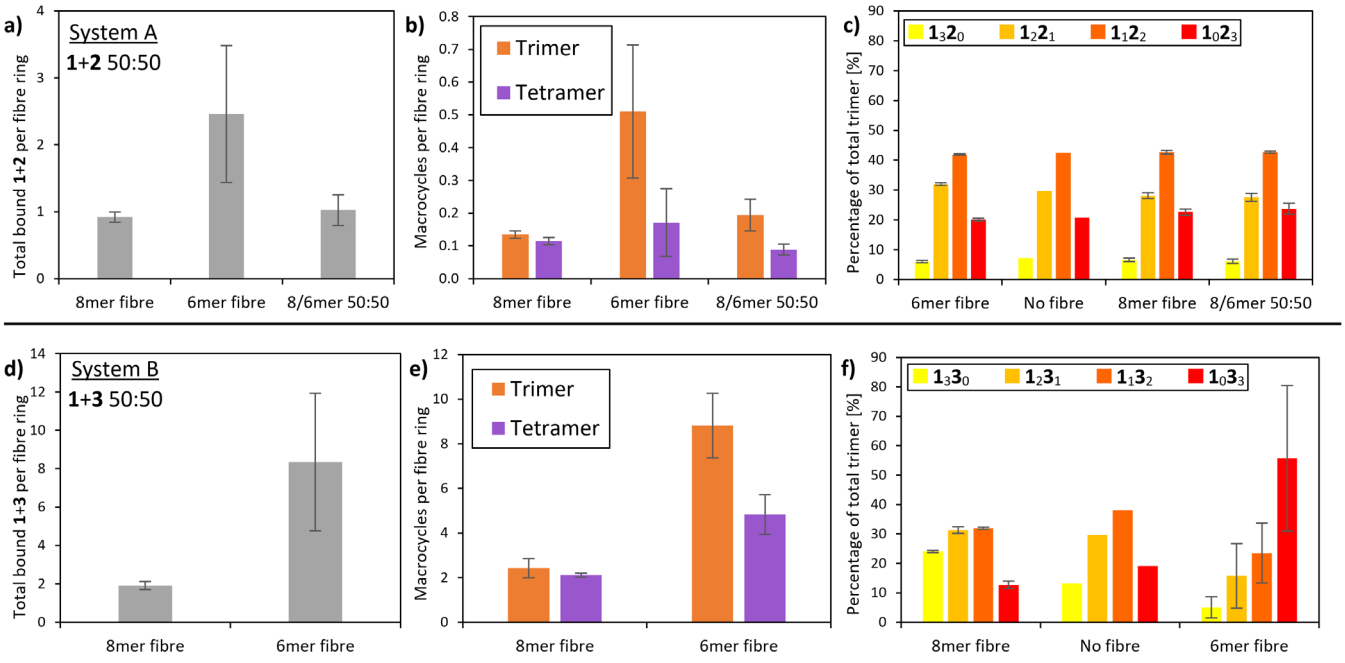

Figure 7. Analysis of precursor material bound on fibres after centrifugation experiments (panels (a-c): system A, (d-f): system B). (a+d) Amount of total material bound on fibres (building block units per fibre ring); (b+e) Amount of trimers and tetramers bound on fibres (number of macrocycles per fibre ring); (c+f) Isomeric distribution of bound trimers (isomer percentage of total bound trimers). Fibres $(\mathbf{1}+\mathbf{2})_8$, $(\mathbf{1}+\mathbf{2})_6$, $\mathbf{1}_8$ *or* $\mathbf{3}_6$ *were added to the corresponding precursor solution, centrifuged and the resulting pellet (containing fibre + bound precursors) analysed by UPLC. "No fibre" entries correspond to analyses of the precursor solution prior to fibre addition. Note: due to peak overlap (cf. SI section II.2.2), in System A the total amount of precursor bound to hexamers is probably underestimated (panel a).*

The fact that system A exhibits no building block preference in precursor binding is consistent with building blocks **1** and **2** being more similar in hydrophobicity than **1** and **3** (reflected in reversed-phase-UPLC retention times, cf. SI section III.1). With precursor binding being non-selective in this system, the dominance of octamer replicator must have a kinetic origin. We

monitored the rate of replication of the hexamer and octamer in system A separately, starting from pre-formed replicator. The conditions were identical to those used for the serial transfer experiments in Figure 4, except that the buffer concentration was raised from 50 to 200 mM (serial transfer in these conditions gave comparable hexamer exclusion, cf. Figure S9) to speed up fibre growth. Individually the octamer (Figure 8a) and hexamer (Figure 8b) replicated with comparable rates and the dominance of octamer in system A cannot be explained by a difference in individual growth rate. Instead, it appears that hexamer and octamer fibres interfere with each other's growth, as apparent from a kinetic experiment in which both replicators were present from the start. In this case the hexamer grows fast over the first three hours and then stops (Figure 8c). In contrast, the octamer grows steadily, albeit substantially slower than in the absence of hexamer, to constitute 77% of the DCL after ca. 70 h (hexamer: 22%; not shown). Note that this behaviour is different from the parallel growth of the same two replicators observed in an earlier study, where different conditions were used.[32] An investigation of the impact of reaction conditions is described in the SI, section I.7.1 (Figures S10-S22).

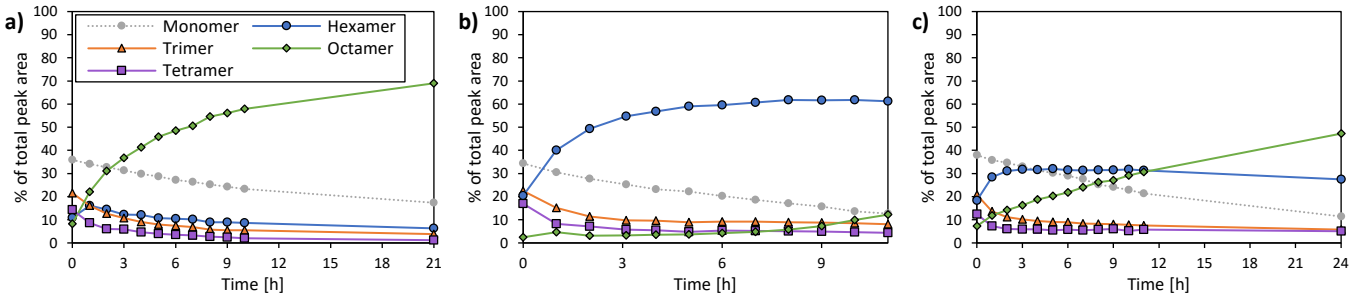

Figure 8. Replication kinetics in system A (**1**+**2**). Growth of (a) octamer replicator starting from 10 mol% octamer; (b) hexamer replicator starting from 10 mol% hexamer and (c) octa- and hexamer replicators starting from a mixture of 5 mol% octamer and 5 mol% hexamer. Conditions: 1.0 mM **1**+**2** (50:50), 50% pre-oxidation by $NaBO_3$, 200 mM borate buffer. Notes: the hexamers are overestimated by 5-10% in the early stage of the reaction (cf. Materials & Methods section II.2.2); in panel b, the octamer nucleates spontaneously (growth starting after 6 h) which might influence the growth of the hexamer.

A separate experiment, in which hexamer growth was monitored, starting from hexamer only, confirmed that the addition of 5 mol% octamer fibres (at t = 3 h) slowed down hexamer growth (Figure S12). We ascribe these effects to interactions between the fibres of the different replicators interfering with the access that these replicators have to bound precursors. This hypothesis is supported by TEM analyses of fibres obtained during serial transfer: hexamer and octamer fibres were found to form bundles with distinct morphologies (SI section I.8, Figure S23; cf. also an earlier study[32]). These persist when both are mixed post-growth. However, in a sample in which octa- and hexamers grew together we can no longer distinguish two distinct morphologies, while fibres are still laterally associated. These observations suggest that hexamer and octamer fibres bind to each other. We probed how this association affects precursor binding using centrifugation experiments. When separate, hexamer fibres bind more precursor than octamer fibres. When grown together, a sample containing both octamer and hexamer in a 50:50 ratio was found to bind only a small amount of precursor, comparable to an octamer-only sample (Figure 7a). The ratio of trimers and tetramer in the bound precursors was 2:1, which is in between the corresponding ratio observed for the individual fibres (Figure 7b). Thus, a possible explanation for the replication kinetics in Figure 8a is that aggregation of both fibres leads to a reduced access to food, which affects especially the hexamer, causing its replication to stall – possibly because it is located more towards the inside of the fibre assemblies, once a substantial amount of octamer has been formed. The higher tendency of hexamer to bind to precursors could also cause it to be bind to, and become surrounded by, octamer fibres. In addition, fibre growth was observed to depend on the building-block composition of the precursor material. The hexamer growth rate was found to be slower at a higher **1**/**2** ratio, while octamer growth rate is not affected by the **1**/**2** ratio. Note that this ratio increases over the course of the reaction as the uptake of **2** by both octa- and hexamer replicators is initially faster than of **1** (SI section I.7.3). This effect might contribute to the stalling of hexamer growth, even more since the depletion of **2** from the precursor material seems to be enhanced by octamer/hexamer interaction. However, the importance of the changing **1**/**2** ratio for the dominance of octamer is likely to be relatively small, since the **1**/**2** ratio in the final octamer and hexamer replicator fibres, where these grew side by sides, exhibit only minor differences (see Figure 3).

We speculate that the fact that competitive exclusion occurs in system A, while coexistence takes place in system B, is due to the bigger structural difference between building block **1** and **3** compared to **1** versus **2**. Building block **3** is more hydrophobic than **2** (also reflected in its later elution from the reversed-phase UPLC column) and therefore more distinct from building block **1**. This allows the replicator fibres in system B to distinguish between the two and preferentially bind one of them, while such discrimination fails to take place in system A.

Conclusion

We have shown that the competitive exclusion principle can be used to curtail diversity in two different systems in which three self-replicator quasi-species emerge with different macrocycle sizes: pentamers, hexamers and octamers, which compete for two common building blocks. In the first system all self-replicators incorporate both building block resources in similar amounts, which causes them to all occupy the same niche. When subjected to a replication-destruction regime, implemented by serial transfer, only the octamer population prevails at the expense of the hexamer and pentamer quasi-species, irrespective of their initial ratios. Mechanistic investigations suggest that the octamer replicator fibres bind to their competitors, hampering the ability of the latter to bind precursor material. As the winning sub-set of replicators in a mutation-selection balance[24,31] the octamer population can be referred to as a quasi-species. In the second system, the pentamer and hexamer populations preferentially incorporate one of the building blocks while the octamer quasi-species preferentially incorporates the other. This selectivity was found to exist already at the stage where the replicators bind to precursor material, with the two building blocks most likely exhibiting different affinities for the surfaces of the different fibres. Thus, the pentamer and hexamer populations occupy the same niche, while the octamer occupies a different one. In serial transfer experiments the hexamer populations were found to competitively exclude the pentamers while stably co-existing with the octamer populations. These results show that the principles of competitive exclusion and resource partitioning, that play a key role in biology, also extend to abiotic chemical systems of fully synthetic replicators. The competitive exclusion principle provides a means for taming the inherent tendency for chemistry to diversify, reducing diversity in cases where different replicators occupy the same niche. At the same time, resource partitioning provides a means to avoid limiting diversity too much. Diversity, in the form of stably coexisting communities of replicators, is a promising starting point for overcoming Eigen's paradox, a major remaining hurdle in the transition from chemistry into biology. Overcoming this hurdle requires that replicator communities cooperate,[36–38] such that selection can act on the collective. Among the different ways such cooperation can be achieved, metabolic coupling seems particularly promising, given the recent discovery of individual replicators capable of sustaining a primitive metabolism[39–41] and given the encouraging results obtained through modelling the evolutionary dynamics of such systems.[28,42] It appears that the pieces of the puzzle of the emergence of life are starting to come together.

Author information

Corresponding Author

Sijbren Otto – Centre for Systems Chemistry, Stratingh Institute, University of Groningen 9747 AG Groningen, The Netherlands; orcid.org/0000-0003-0259-5637; Email: s.otto@rug.nl

Present Addresses

Marcel J. Eleveld – Centre for Systems Chemistry, Stratingh Institute, University of Groningen 9747 AG Groningen, The Netherlands; orcid.org/0000-0001-6388-0461
Yannick Geiger – Centre for Systems Chemistry, Stratingh Institute, University of Groningen 9747 AG Groningen, The Netherlands; orcid.org/0000-0003-2280-7107
Juntian Wu – Centre for Systems Chemistry, Stratingh Institute, University of Groningen 9747 AG Groningen, The Netherlands; orcid.org/0000-0003-3894-9812
Armin Kiani – Centre for Systems Chemistry, Stratingh Institute, University of Groningen, 9747 AG Groningen, The Netherlands; orcid.org/0000-0002-2744-4627
Gaël Schaeffer – Centre for Systems Chemistry, Stratingh Institute, University of Groningen 9747 AG Groningen, The Netherlands; orcid.org/0000-0001-9375-399X

Author contributions

MJE, GS and SO conceptualized the study. Experimental work was designed, performed and resulting data analysed by AK (TEM), YG & JW (mechanistic investigations), JW (redox infusion) and MJE (all other experimental work). MJE, YG and SO wrote the manuscript.

† YG & JW contributed equally to this work.

Data availability statement

The raw data generated during this study is available from the corresponding author upon reasonable request.

Acknowledgements

This research was supported by the ERC (AdG 741774), NWO (VICI grant) and Zernike Dieptestrategie, the Dutch Ministry of Education, Culture and Science (Gravitation Program 024.001.035), the China Scholarship Council and from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 847675 (oLife post-doctoral fellowship programme).

Competing interests

The authors declare no competing interests.

References

1. Adamski, P. *et al.* From self-replication to replicator systems en route to de novo life. *Nat. Rev. Chem.* **4**, 386–403 (2020).
2. Otto, S. An Approach to the De Novo Synthesis of Life. *Acc. Chem. Res.* **55**, 145–155 (2022).
3. von Kiedrowski, G. Minimal Replicator Theory I: Parabolic Versus Exponential Growth. in *Bioorganic Chemistry Frontiers* (eds. Dugas, H. & Schmidtchen, F. P.) vol. 3 113–146 (Springer Berlin Heidelberg, 1993).
4. Szathmáry, E. & Gladkih, I. Sub-exponential growth and coexistence of non-enzymatically replicating templates. *J. Theor. Biol.* **138**, 55–58 (1989).
5. Carnall, J. M. A. *et al.* Mechanosensitive Self-Replication Driven by Self-Organization. *Science* **327**, 1502–1506 (2010).
6. Rubinov, B., Wagner, N., Rapaport, H. & Ashkenasy, G. Self-Replicating Amphiphilic β-Sheet Peptides. *Angew. Chem. Int. Ed.* **48**, 6683–6686 (2009).
7. Luther, A., Brandsch, R. & von Kiedrowski, G. Surface-promoted replication and exponential amplification of DNA analogues. *Nature* **396**, 245–248 (1998).
8. Colomb-Delsuc, M., Mattia, E., Sadownik, J. W. & Otto, S. Exponential self-replication enabled through a fibre elongation/breakage mechanism. *Nat. Commun.* **6**, 7427 (2015).
9. Hordijk, W., Vaidya, N. & Lehman, N. Serial transfer can aid the evolution of autocatalytic sets. *J. Syst. Chem.* **5**, 4 (2014).
10. Vaidya, N. *et al.* Spontaneous network formation among cooperative RNA replicators. *Nature* **491**, 72–77 (2012).
11. Lifson, S. & Lifson, H. Coexistence and Darwinian Selection among Replicators: Response to the Preceding Paper by Scheuring and Szathmary. *J. Theor. Biol.* **212**, 107–109 (2001).
12. Lincoln, T. A. & Joyce, G. F. Self-Sustained Replication of an RNA Enzyme. *Science* **323**, 1229–1232 (2009).
13. Bandela, A. K. *et al.* Primitive selection of the fittest emerging through functional synergy in nucleopeptide networks. *Proc. Natl. Acad. Sci. U. S. A.* **118**, e2015285118 (2021).
14. Yang, S. *et al.* Chemical Fueling Enables Molecular Complexification of Self-Replicators. *Angew. Chem. Int. Ed.* **60**, 11344–11349 (2021).
15. Pascal, R., Pross, A. & Sutherland, J. D. Towards an evolutionary theory of the origin of life based on kinetics and thermodynamics. *Open Biol.* **3**, 130156 (2013).
16. Pross, A. & Pross, A. *What is Life?: How Chemistry Becomes Biology*. (Oxford University Press, 2016).
17. Gause, G. F. Experimental Studies on the Struggle for Existence: I. Mixed Population of Two Species of Yeast. *Journal of Experimental Biology* **9**, 389–402 (1932).

18. Hardin, G. The Competitive Exclusion Principle. *Science* **131**, 1292–1297 (1960).
19. Connell, J. H. The Influence of Interspecific Competition and Other Factors on the Distribution of the Barnacle Chthamalus Stellatus. *Ecology* **42**, 710–723 (1961).
20. Schoener, T. W. Resource Partitioning in Ecological Communities. *Science* **185**, 27–39 (1974).
21. McKane, R. B. *et al.* Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* **415**, 68–71 (2002).
22. Levine, J. M. & HilleRisLambers, J. The importance of niches for the maintenance of species diversity. *Nature* **461**, 254–257 (2009).
23. Szilágyi, A., Zachar, I. & Szathmáry, E. Gause's Principle and the Effect of Resource Partitioning on the Dynamical Coexistence of Replicating Templates. *PLoS Comput. Biol.* **9**, e1003193 (2013).
24. Eigen, M. Selforganization of matter and the evolution of biological macromolecules. *Naturwissenschaften* **58**, 465–523 (1971).
25. Eigen, M. & Schuster, P. A principle of natural self-organization. *Naturwissenschaften* **64**, 541–565 (1977).
26. Wilson, D. S. A theory of group selection. *Proc. Natl. Acad. Sci. U. S. A.* **72**, 143–146 (1975).
27. Szathmáry, E. & Demeter, L. Group Selection of Early Replicators and the Origin of Life. *J. Theor. Biol.* **128**, 463–486 (1987).
28. Czárán, T., Könnyu, B. & Szathmáry, E. Metabolically Coupled Replicator Systems: Overview of an RNA-world model concept of prebiotic evolution on mineral surfaces. *J. Theor. Biol.* **381**, 39–54 (2015).
29. Malakoutikhah, M. *et al.* Uncovering the Selection Criteria for the Emergence of Multi-Building-Block Replicators from Dynamic Combinatorial Libraries. *J. Am. Chem. Soc.* **135**, 18406–18417 (2013).
30. Frederix, P. W. J. M. *et al.* Structural and Spectroscopic Properties of Assemblies of Self-Replicating Peptide Macrocycles. *ACS Nano* **11**, 7858–7868 (2017).
31. Eigen, M. *Steps Towards Life: A Perspective on Evolution*. (Oxford University Press, 1992).
32. Schaeffer, G. *et al.* Stochastic Emergence of Two Distinct Self-Replicators from a Dynamic Combinatorial Library. *J. Am. Chem. Soc.* **144**, 6291–6297 (2022).
33. Blokhuis, A., Lacoste, D. & Gaspard, P. Reaction kinetics in open reactors and serial transfers between closed reactors. *J. Chem. Phys.* **148**, 144902 (2018).
34. Hatai, J. *et al.* An Optical Probe for Real-Time Monitoring of Self-Replicator Emergence and Distinguishing between Replicators. *J. Am. Chem. Soc.* **144**, 3074–3082 (2022).

35. Maity, S. *et al.* Caught in the Act: Mechanistic Insight into Supramolecular Polymerization-Driven Self-Replication from Real-Time Visualization. *J. Am. Chem. Soc.* **142**, 13709–13717 (2020).

36. Mathis, C., Ramprasad, S. N., Walker, S. I. & Lehman, N. Prebiotic RNA Network Formation: A Taxonomy of Molecular Cooperation. *Life* **7**, 38 (2017).

37. Yeates, J. A. M., Nghe, P. & Lehman, N. Topological and thermodynamic factors that influence the evolution of small networks of catalytic RNA species. *RNA* **23**, 1088–1096 (2017).

38. Lee, D. H., Severin, K., Yokobayashi, Y. & Ghadiri, M. R. Emergence of symbiosis in peptide self-replication through a hypercyclic network. *Nature* **390**, 591–594 (1997).

39. Monreal Santiago, G., Liu, K., Browne, W. R. & Otto, S. Emergence of light-driven protometabolism on recruitment of a photocatalytic cofactor by a self-replicator. *Nat. Chem.* **12**, 603–607 (2020).

40. Ottelé, J., Hussain, A. S., Mayer, C. & Otto, S. Chance emergence of catalytic activity and promiscuity in a self-replicator. *Nat. Catal.* **3**, 547–553 (2020).

41. Arsène, S., Ameta, S., Lehman, N., Griffiths, A. D. & Nghe, P. Coupled catabolism and anabolism in autocatalytic RNA sets. *Nucleic Acids Res.* **46**, 9660–9666 (2018).

42. Szilágyi, A., Könnyű, B. & Czárán, T. Dynamics and stability in prebiotic information integration: an RNA World model from first principles. *Sci. Rep.* **10**, 51 (2020).