15. Jayaraman, S., Fausey, C.M., and Smith, L.B. (2017). Why are faces denser in the visual experiences of younger than older infants? Dev. Psychol. *53*, 38–49.

16. Fausey, C.M., Jayaraman, S., and Smith, L.B. (2016). From faces to hands: changing visual input in the first two years. Cognition *152*, 101–107.

17. Franchak, J.M., Kretch, K.S., Soska, K.C., and Adolph, K.E. (2011). Head-mounted eye tracking: a new method to describe infant looking. Child Dev. *82*, 1738–1750.

18. Kretch, K.S., Franchak, J.M., and Adolph, K.E. (2014). Crawling and walking infants see the world differently. Child Dev. *85*, 1503–1518.

19. Smith, L.B., Yu, C., and Pereira, A.F. (2011). Not your mother's view: the dynamics of toddler visual experience. Dev. Sci. *14*, 9–17.

20. Rogers, S.J., and Dawson, G. (2010). Early Start Denver Model for Young Children with Autism: Promoting Language, Learning and Engagement (New York: Guilford Press).

In vitro evolution: From monsters to mobs

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https://doi.org/10.1016/j.cub.2022.05.008

During an *in vitro* evolution experiment over hundreds of generations, a replicator system, begun with a single RNA species and the replicase it encodes, spontaneously generated a multi-member network where parasitism, altruism, and the environment play key roles.

Practitioners of synthetic biology often draw inspiration from Richard Feynman's famed chalkboard writing: "What I cannot create, I do not understand." As a theoretical physicist might create a step-by-step derivation to explain a natural phenomenon, synthetic biologists of a certain stripe create at first simple, and then increasingly complex, molecular assemblies to coax life-like properties from nonliving matter. Such step-wise studies allow one to capture the moment when new behaviors emerge, and thus explain them by a minimal number of components and interactions. In a new study, while evolving a simple enzymatic replicator system *in vitro*, Ryo Mizuuchi and colleagues observed the spontaneous emergence of an interdependent molecular network[1].

The first demonstration that life-associated molecules could be synthesized without a cellular or biological system can be traced back to 1828, when Fredrich Wöhler showed that urea can be synthesized from ammonium cyanate "without the need for an animal kidney"[2]. One of the simplest possible evolvable systems was developed a century and a half later, when Sol Spiegelman concocted a recipe for molecular replication *in vitro*, using the RNA genome of phage Qβ along with the Qβ replicase, the phage enzyme that recognizes the Qβ genome and makes an RNA copy[3]. This minimal system exhibited replication, mutation through replicase errors, and selection based on genetically inherited traits. The incorporation of environmental constraints and selection pressures thus created a synthetic system to study the *sine qua non* of biological systems: Darwinian evolution[4].

One of the earliest insights from the Qβ replicase experiments was that selection does not always favor biological function or complexity. Reducing incubation times, while continuing to supply ample wild-type Qβ replicase, favored shorter and shorter RNA sequences, ultimately giving rise to Spiegelman's 'monsters' — easily replicated sequences that had discarded any genetic information aside from the replicase recognition signal[3]. Such mutants arose through recombination that generated genomic deletions. Although highly fit in the presence of Qβ replicase activity, these 'monsters' essentially parasitized wild-type Qβ replicase, and could not produce functional Qβ replicase themselves. Thus, the Qβ system posed a fundamental question in synthetic biology: what modifications to the system would maintain, or even favor, increased function and complexity?

In recent years, the Qβ replicator system has been adopted by additional researchers, advancing the complexity of the system while bringing modern technologies to bear. The nature of the system changed fundamentally when the ability to translate proteins was added by incorporating a cell-free translation mix with the Qβ replicator system; referred to as a translation-coupled RNA replication (TcRR) system (Figure 1A). This modification eliminated the need to add wild-type Qβ replicase to successive generations. More importantly, the ability to translate proteins created a feedback mechanism in which the newly evolved Qβ genomes would encode the replicases for the next generation of genome replication. In the TcRR system, without wild-type Qβ replicase to parasitize, Spiegelman's monsters suffered from their own loss of genetic information, and replication ground to a halt; the monsters overran the environment that supported them. However, the fate of this system could be rescued by an ecological change: compartmentalization, created using water-in-oil droplets[5] (Figure 1B). Monsters still arose by mutation and consumed the resources in their droplet, but compartmentalization confined those newly arisen monsters within their own droplets during that round. At the same

time, droplets that lacked monsters were able to produce many copies of functional Qβ replicase along with the encoding RNA. When fresh reagent droplets were added and fused, and new droplets created for the next generation, the genomes producing functional replicase could persist despite the spontaneous emergence of monsters[6]. That is, although selection within an individual droplet still favored parasites, droplets without parasites created more descendants than droplets with parasites, resulting in a form of 'group' selection that countered the tendency for the system to lose activity to monsters[7,8]. Furthermore, when observed over more than a hundred serial transfer generations, the Qβ replicases and parasites showed signs of a co-evolutionary arms race: the replicases evolved some resistance to the parasites, showing reduced preference for the parasites' recognition sequence, and then in turn the parasites evolved an expanded recognition signal improving their recognition by the replicases[9]. These earlier studies showed how the replicase activity could be maintained in the TcRR Qβ system despite the evolution of Spiegelman's monsters and, more generally, how a very simple *in vitro* replicator system could dynamically sustain its own complexity. However, until recently, the emergence of greater complexity, or growth of the network itself, had remained elusive.

In recent work, Mizuuchi and collaborators[1] described how, when continuing to propagate the compartmentalized TcRR Qβ system over hundreds of generations, several different lineages of hosts and parasites came to co-exist in the system. After 240 generations, the system consisted of 5 different lineages (3 replicase lineages and 2 parasite lineages). The co-existence of multiple lineages itself is not necessarily surprising; at a high enough mutation rate, the succession of different mutants sweeping through the population can overlap one another, giving a diversified mixture. However, an examination of the phenotypes of the lineages over time revealed a surprising dynamic of increasing 'altruism' in one lineage (host lineage 2, or HL2). This type of behavior indicates the emergence of cooperation, at a molecular level, that can generate new levels of complexity[10]. Initially, the replicase lineages (at first, just two

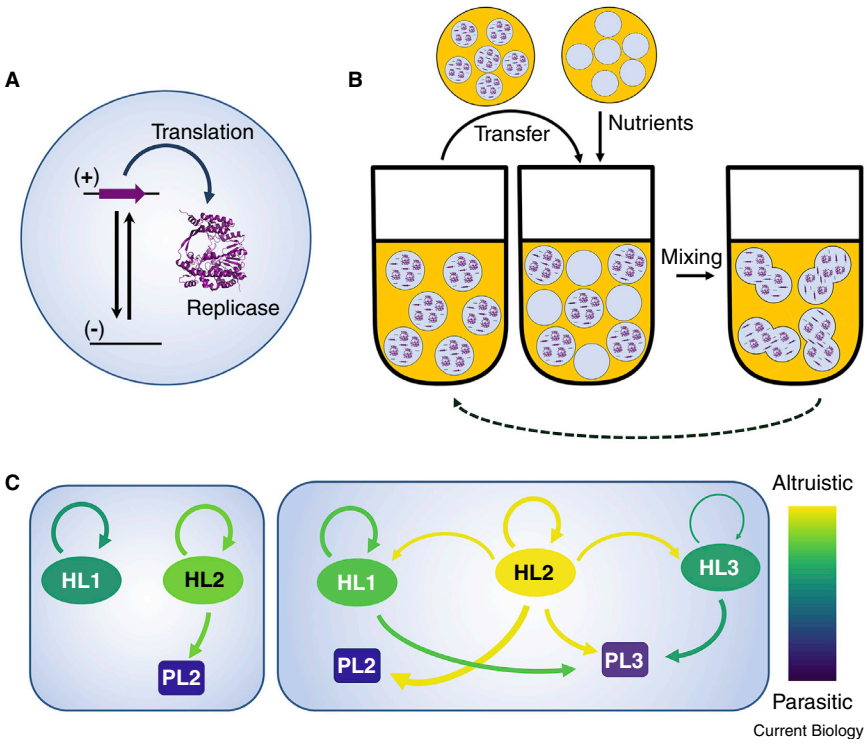

Figure 1. Evolution of a network of replicase-encoding RNAs and their corresponding enzymes. (A) Schematic representation of the TcRR Qβ system. The plus strand of RNA is translated into a replicase enzyme, which catalyzes the copying of (+)-RNA into reverse-complementary (-)-RNA, and vice versa. (B) Serial transfer of droplets to create evolutionary generations in the compartmentalized TcRR system. Droplets containing RNA and replicases are 'fed' with nutrient-containing droplets, mixed, and reformed into new droplets. (C) Depiction of interactions early (left) and late (right) in the experiment. Arrow thickness indicates relative replication activity. Initially isolated systems (HL1 and HL2/PL2) evolved into an interdependent network. (The three-dimensional structure of the replicase shown in (A) was based on Protein Data Bank entry 3MMP[16].)

lineages, HL1 and HL2) lacked the ability to replicate one another, and replicase HL2 also replicated a parasite (parasite lineage 2, or PL2). A descendent variant of HL2, called HL3, then emerged. HL3 was a fairly poor replicase, having only weak activity to replicate itself and HL2, and mostly relied on replication by the parental HL2 type. Indeed, HL3 had only a short lifetime during the experiment and did not prove to be a stable member of the network. At this point, only HL1 was not able to utilize HL2, but this ability soon emerged, resulting in a network where HL2 replicated all of the other RNAs. As for the parasites, PL2 gave rise to a mutant type PL3, which parasitized HL1, HL2 and HL3 approximately evenly. PL2 itself lost the ability to parasitize HL3, but made up for this loss with increased parasitization of HL2. By the end of the experiment, all of the replicases could interact with multiple types of RNA molecule (replicase or parasite), forming a network of replicators (Figure 1C), where HL2 demonstrated a notable ability to replicate all other RNAs. Yet aside from HL2 itself, no other RNAs could replicate HL2. Overall, not only was the TcRR Qβ system able to resist a takeover by parasites, exemplified by the altruistic HL2, but the system also exhibited spontaneous expansion as well as merging of initially distinct networks (replicated by HL1 and HL2) into a single network with a general replicase.

A fascinating property of the network, demonstrated through computer simulation, was that removal of one component would cause the extinction of at least one other member of the network. While the removal of a replicase could certainly eliminate a parasite (that is, removing HL2 would eliminate its specialist parasite PL2, and removing HL1 might decrease the production of generalist parasite PL3 sufficiently to cause its extinction), why should removal of a *parasite* cause extinction of a

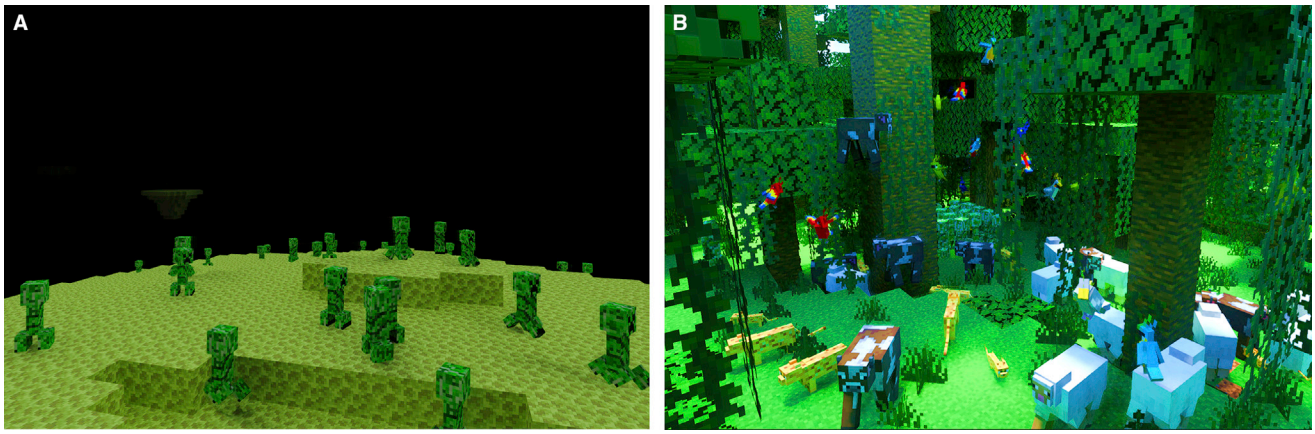

Figure 2. Monsters or mobs?
Whereas some environments allow takeover by parasitic 'monsters' (A), others promote the evolution and co-existence of multiple interacting species (B). (Image credit: B. Schindlinger; illustration created using Minecraft Bedrock edition 1.18 with Defined PBR RTX.)

replicase? In particular, removal of parasite PL2, which was only replicated by HL2, was observed to cause extinction of replicase HL1. To understand this, one may consider that removal of PL2 frees up HL2 to increase replication of all other RNAs, including generalist parasite PL3. With more PL3 in the system, more HL1 would become occupied with replicating PL3, reducing the amount of HL1 self-replication enough to cause its eventual disappearance (that is, a reproductive ratio less than 1). Perhaps even more surprisingly, removal of parasite PL3 was observed to cause extinction of the general replicase HL2. In this case, without PL3, all HL1 replicase activity would be directed toward self-replication, allowing HL1 to crowd out HL2. With the disappearance of HL2, extinction of PL2 soon followed, leaving only HL1 in the system. Thus, the parasites help preserve the network by keeping certain replicases in check and preventing overgrowth, which also kept other parasites in check, preventing them from overtaxing the replicases. This final network, though small, demonstrates a discontinuity in complexity: although a subsystem having one or two members, or a full network of four or five members, might be stable, removing members to form any three-member network would create an unstable, short-lived system. Such discontinuous increments lie at the heart of biological complexity.

An important question is whether the observed phenomenon, the emergence of a larger network from individual replicases, would be a rare fluke or a common event[11]. Additional experimental replicates by the authors showed that, although the specific genotypes of replicases and parasites evolved in the last rounds depended on the historical replicate, the final outcome — formation of networks — was repeatedly observed. These results are consistent with some of the findings of the long-term evolution experiment by Richard Lenski and his colleagues[12], in which spontaneous emergence of ecological interactions appeared consistently during long-term adaptation of *E. coli* populations to a particular environment[13]. In another context, interdependent networks were also observed to emerge within a diverse pool of recombination ribozymes[14]. In the TcRR Qβ system, with compartmentalization to prevent parasites from taking over and a population size and mutation rate sufficient to generate diversity in the population, the emergence of a network appears to be a common result. Continued development and experimental evolution of this increasingly complex system, perhaps in new environments, promises to be a fascinating endeavor.

Thus, while some environments cause Spiegelman's monsters to dominate the Qβ system, leading to a tragedy of the commons, more complex environments can promote the emergence of networks with multiple members. Much like the 'mobs' (animals and monsters) of the popular video game Minecraft, these members form both positive and negative relationships with each other in their new ecosystem[15] (Figure 2). The emergence of molecular mobs in this simple replicase system illustrates one of the fundamental aspirations of synthetic biology — to create life-like systems, and in so doing, to begin to understand biological complexity.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Mizuuchi, R., Furubayashi, T., and Ichihashi, N. (2022). Evolutionary transition from a single RNA replicator to a multiple replicator network. Nat. Commun. *13*, 1460.
2. Leicester, H.M. (1974). Development of Biochemical Concepts from Ancient to Modern Times (Cambridge: Harvard University Press).
3. Mills, D.R., Peterson, R.L., and Spiegelman, S. (1967). An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule. Proc. Natl. Acad. Sci. USA *58*, 217–224.
4. Joyce, G.F. (2007). Forty years of *in vitro* evolution. Angew. Chem. Int. Ed. Engl. *46*, 6420–6436.
5. Ichihashi, N., Usui, K., Kazuta, Y., Sunami, T., Matsuura, T., and Yomo, T. (2013). Darwinian evolution in a translation-coupled RNA replication system within a cell-like compartment. Nat. Commun. *4*, 2494.
6. Bansho, Y., Furubayashi, T., Ichihashi, N., and Yomo, T. (2016). Host-parasite oscillation dynamics and evolution in a compartmentalized RNA replication system. Proc. Natl. Acad. Sci. USA *113*, 4045–4050.
7. Wilson, D.S. (1975). A theory of group selection. Proc. Natl. Acad. Sci. USA *72*, 143–146.
8. Szathmáry, E., and Demeter, L. (1987). Group selection of early replicators and the origin of life. J. Theor. Biol. *128*, 463–486.
9. Furubayashi, T., Ueda, K., Bansho, Y., Motooka, D., Nakamura, S., Mizuuchi, R., and Ichihashi, N. (2020). Emergence and diversification of a host-parasite RNA ecosystem through Darwinian evolution. eLife *9*, e56038.

10. Nowak, M.A. (2006). Five rules for the evolution of cooperation. Science *314*, 1560–1563.

11. Lenski, R.E. (2017). Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. ISME J. *11*, 2181–2194.

12. Blount, Z.D., Lenski, R.E., and Losos, J.B. (2018). Contingency and determinism in evolution: Replaying life's tape. Science *362*, eaam5979.

13. Good, B.H., McDonald, M.J., Barrick, J.E., Lenski, R.E., and Desai, M.M. (2017). The dynamics of molecular evolution over 60,000 generations. Nature *551*, 45–50.

14. Vaidya, N., Manapat, M.L., Chen, I.A., Xulvi-Brunet, R., Hayden, E.J., and Lehman, N. (2012). Spontaneous network formation among cooperative RNA replicators. Nature *491*, 72–77.

15. Wiltshire, A. (2017). Minecraft Mobestiary. An Illustrated Guide to the Mobs of Minecraft (Copenhagen: Egmont Books).

16. Kidmose, R.T., Vasiliev, N.N., Chetverin, A.B., Andersen, G.R., and Knudsen, C.R. (2010). Structure of the Qbeta replicase, an RNA-dependent RNA polymerase consisting of viral and host proteins. Proc. Natl. Acad. Sci. USA *107*, 10884–10889.

Pathogenesis: How a killer fungus targets its host

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https://doi.org/10.1016/j.cub.2022.05.004

The cellular signals that trigger pathogen recognition of its target hosts are critical components in the infection cycle. A new study describes the amphibian host cues that induce spore germination in the deadly pathogen that causes chytridiomycosis.

Emerging diseases are a major threat to global biodiversity, food security, and human health[1,2]. A better understanding of disease progression at the cellular level can help inform our mitigation of the emerging disease threats we see today. Amphibian chytridiomycosis is the emerging infectious disease responsible for the recent die-offs and extinctions of frog and salamander species worldwide[3]. The chytrid pathogens that cause chytridiomycosis have proven enigmatic since the discovery of *Bd* (*Batrachochytrium dendrobatidis*) in 1998[3,4]. This is because amphibian chytrids belong to a poorly studied phylum of fungi and the amphibian hosts are themselves often cryptic and thus challenging to sample. In this issue of *Current Biology*, Robinson *et al.*[5] describe a compelling series of experiments demonstrating the role of amphibian mucus in triggering spore encystation, a key step in the *Bd* infection process.

Understanding the emergence and spread of the amphibian-killing chytrid fungus *Bd* has been a key research interest since it was first confirmed as the cause of the alarming frog declines at the turn of the last century. To understand the emergence of novel diseases, ecologists traditionally conceptualize the three major factors contributing to disease as a theoretical triangle — the so-called 'disease triangle'[6]. The three corners of this model represent the three interacting factors necessary for disease to develop: a susceptible host, a pathogen capable of attack, and a favorable environment for infection. The principle of this framework is that all three factors must be present for disease conditions to manifest.

Research on chytridiomycosis over the last two decades has focused intensely on two corners of the disease triangle: host factors influencing susceptibility and environmental factors contributing to disease. We are now increasingly able to predict the environmental conditions favorable for the emergence and development of chytridiomycosis, from fine-scale, environmental correlates of chytridiomycosis prevalence[7,8], to robust global-scale predictions of regions that have potential hotspots for disease outbreaks[9]. Likewise, our understanding of amphibian defenses has grown by leaps and bounds over the last two decades. We now have good working models of how amphibian immunity responds to *Bd* infection[10] and the role that the skin microbiome plays in defense[11]. Importantly, we also have a better understanding of how amphibian populations evolve resistance over time[12].

In contrast to our understanding of the environmental and host factors contributing to disease, fewer studies exist that examine the third critical component of the disease triangle — factors regulating the pathogen's capacity to attack amphibians. Without this missing, fundamental piece of the disease puzzle, we are faced with a gap in our ability to fully understand (and control) the current global spread of amphibian-killing chytrids.

Like most other chytrid species in the phylum Chytridiomycota, *Bd* (and its genus *Batrachochytrium*) remain poorly understood. Chytrids are among the most anciently diverging lineages of fungi and the extent of biodiversity in this group of fungi is still under active exploration. Interestingly, chytrids retain the ancestral character of dispersing by motile, swimming spores called zoospores. The zoospores of chytrid species propel themselves through water with a single posterior flagellum — a deep, conserved homology shared with their evolutionary cousins, animal sperm cells[13]. These swimming, or sometimes crawling, zoospores are capable of dispersing over short distances in aquatic environments to seek a susceptible host cell.

A key stage in the *Bd* life cycle occurs when a zoospore encounters a suitable