colleagues' technique[1] is the sequential control over a self-assembly process that involves different interactions — first hydrophobic forces, then electrostatic repulsion and finally van der Waals attraction. Sequential self-assembly has been carried out before, most notably in beautiful experiments by Ned Seeman[6] and Chad Mirkin[7] and their colleagues using artificial sequences of DNA. But those experiments use the coding of DNA base pairs rather than different forces to control the sequence of self-assembly. Moreover, they are 'biokleptic' (Seeman's term): that is, they borrow heavily from biological processes. Onoe and colleagues' approach is more general and, at the current stage of development, much less powerful. It does, however, represent a useful addition to our toolbox.

To develop self-assembly into a practical technology, we will need to be even more ingenious than nature, exploiting all the interactions at our disposal and creating yet-to-be envisaged pathways. The recent work[1] takes us a step further down that road. ■

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EVOLUTIONARY BIOLOGY

Structure in mutualistic networks

Susanne S. Renner

Statistical analyses of the networks formed by plant–animal mutualisms can now take account of the relatedness of the players on either side. How helpful is this innovation for understanding network dynamics?

The mutually beneficial relationships between plants and animals take several forms. One example is pollination. Another is the process by which a fruit-eating creature, a frugivore, gets a meal and subsequently disperses a plant's seeds in its droppings.

In the context of a local ecological community, such relationships can be seen as networks in which the evolutionary dynamics of the partners may be mutually dependent, contributing to an array of coevolutionary processes[1].

On page 925 of this issue, Rezende *et al.*[2] report an analysis of plant–pollinator and plant–frugivore networks that includes information on the evolutionary history of the partners — that is, on the phylogenetic relatedness of each partner to other plants or animals in the network. Their aim was to determine whether relatedness affects network structure and whether it predicts 'cascades' of coextinction. This is the first such evolutionary network analysis, and it highlights both the power and the limitations of the approach. Two central concepts are those of species 'strength' and species 'degree' (as described in Box 1, overleaf, which gives the background to the method). Combining the two concepts has produced a boom in the analysis of plant–animal networks[2–4].

In mutualistic networks, optional interactions occurring among many species are common; most interactions are strongly asymmetric; and species interact with nested subsets of partners[1,5,6]. The dense core of reciprocal interactions coexists with a relatively small number of super-generalists. Relatively rare plants and animals, as well as those with comparatively few partners, interact primarily with a core group of abundant generalist species.

Related participants in mutualisms are likely to have similar morphology, physiology and behaviour. These are traits that evolve as lineages diversify, so we would expect the structure of mutualistic networks to be influenced by the hierarchical phylogenetic relationships present in a particular community. This is the assumption Rezende *et al.*[2] set out to test. Statistical methods to estimate the role of phylogenies in explaining patterns of trophic (feeding) association are now available[2,7]; these methods are based on established statistics for the phylogenetic comparative method[8,9]. The basic approach is to structure the problem of pollinator–flower associations as a statistical model in which phylogenies are used to give the covariance structure of the 'error' terms.

Rezende *et al.* applied these methods to 59 plant–pollinator and plant–frugivore networks, which were compiled from the literature. Their approach involved obtaining phylogenies for the insects in 35 networks, birds (all frugivores) in 18, and flowering plants in 52. The number of interactions per species was significantly phylogenetically conservative in 25% of the phylogenies (26 of 105) and a third of the networks. Small phylogenies provide little power to detect phylogenetic effects, and when only 22 phylogenies with more than 70 species were considered, this percentage increased to 55%. A correlation between phylogenetic relatedness and ecological similarity — the fraction of common interactors between two species — was detectable in 43% (50 of 103) of phylogenies.

For their ten largest communities (those with more than 40 animal and plant mutualists), Rezende *et al.* simulated coextinction cascades by removing pollinators systematically, starting with the least-linked (most specialized) partners and moving to the most-linked (most generalized). Species left without any local interaction were assumed to become extinct. The simulations show that related species do tend to become extinct together, although the effects were small.

Overall, given the modest percentage correlations cited above, Rezende and colleagues find that phylogenetic relationships do not have a marked effect on the degree and strength of interactions in local communities. This result will disappoint those who expect to find such a signal in every nook and cranny. One explanation may lie in the hugely varied temporal scales over which the hundreds of plant and animal species in the 59 local networks have been interacting. The networks encompass Mediterranean, tropical, temperate, subtropical and Arctic communities, which differ greatly in their stability and numbers of evolutionarily old or recent species. Also, some of the communities have comparatively few closely related species, and so lack a strong phylogenetic structure. Given the different temporal and spatial scales over which different pollinators (birds, bees, flies, beetles) and plants (tropical or temperate, woody or herbaceous) evolve, very large networks may be needed to discern phylogenetic signals in interaction strength and degree[10].

However, even then, phylogeny might not predict numbers and kinds of mutualistic interactions. As has been shown[11] for a network involving many insects and one focal plant, broad-leaved lavender (*Lavandula latifolia*), having few or many visitors to a flower may not be a trait that is invariant at the species level, but instead may depend on research design (sampling effort) and biological phenomena (variation in absolute and relative pollinator abundance or visitation rates). If a large proportion of the interactions counted in pollination networks are not species-level traits, this would explain the absence of clear phylogenetic effects in insect–flower networks.

There is a risk that treating mutualistic networks as "coevolved structures rather than as diffuse multispecific interactions"[5] could lead research on networks into a trap from which community ecology has long escaped[12]. Instead of revealing coevolved interactions, Rezende and colleagues' results might be taken as showing that such interactions are not very important. And in terms of extinctions, the formation of associations between migrating

Box 1 | Methods for quantifying mutualisms

Quantitative approaches to understanding plant–animal mutualisms go back to the application of food-web theory to a large sample of pollination and seed-dispersal networks[13]. Mutualistic interactions can be arranged as a matrix of dependences between $a = 1, 2, ..., i$ species of animal and $p = 1, 2, ..., j$ species of plant, with the total number of species in the system, M, equal to $a + p$.

One can then examine the proportion of all possible interactions that actually occur and, for interacting pairs of species, estimate the relative magnitude of the interaction, or the number of interactions per species (referred to as species 'degree'). The realized number of interactions (connectance) scales in proportion to M; the potential number of interactions scales as $a \times p$; the average number of interactions per species varies independently of M (ref. 13). Because connectance covaries with species richness (as in other food webs), network studies control for M by doing regression analysis on residuals.

Recently, two further parameters have been introduced[5], quantifying mutual dependence or species 'strength'. The first is the dependence of plant species j on animal species i — that is, the fraction of all visits from a particular animal species. The second is the dependence of animal species i on plant species j (that is, the fraction of all visits by this animal species going to a particular plant species). This allows an index of asymmetry to be calculated for each pairwise interaction, paving the way for the latest analyses[2–4]. **S. S. R.**

might actually encourage the persistence of local species in the long term[2,5,10].

Nonetheless, with habitat fragmentation and climate change now occurring so rapidly, mutualistic networks are likely to be severely affected in many places. Setting networks in an evolutionary context might help to predict their level of resilience. Using the approach developed by Rezende *et al.*, future work on networks might incorporate indirect antagonistic interactions affecting mutualistic networks — such as those in which herbivores consume flowers or fruits to the detriment of the plants concerned. ■

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MATERIALS SCIENCE

Embedded shells decalcified

Catherine Picart and Dennis E. Discher

Synthetic microcapsules with membrane-bound inner chambers in which chemical reactions can be isolated and controlled have been assembled, layer by layer. Could artificial cells be on the horizon?

The next time you eat an apple, take a moment to consider its hierarchical structure. The thin, durable skin wraps around the delectable fruit, in which can be found seeds; those seeds, in turn, have a coating of their own to encase their contents. Animal cells possess a similar arrangement of embedded compartments: cell membranes confine the machinery of life, including the nuclei with their membrane-ensconced genomes. Both cell and nuclear membranes self-assemble from lipid molecules, and are fortified with networks of proteins whose structures are templated by interactions with the membranes. The hierar-

ture has now been described in *Angewandte Chemie* by Kreft *et al.*[1]. They have used microspheres of calcium carbonate as templates to construct polymeric, shell-within-shell capsules. Once the structures are assembled, the templates are simply washed away.

The authors adopted a strategy known as layer-by-layer (LbL) assembly, which entails dip-coating a template alternately in two polymer solutions, one of which is positively charged and the other negatively charged. A wide range of polymers can be deposited in this way, to make multilayered structures with an array of physical and chemical properties,

these surface-grown films can coat a broad range of substrates, from large flat surfaces to highly curved colloidal particles. Flexible polymers can be layered onto complex, curved geometries — for example, red blood cells, which have a concave structure, have been used as templates for LbL assembly[3].

Kreft *et al.*[1] used microspheres of ionic calcium carbonate as the template for the inner capsule of their structures. These spheres offer two advantages as templates for LbL assembly. First, they can be dissolved at the end of the process by washing with a solution of EDTA (a molecule that traps calcium ions); this is a milder way of removing templates than previously reported methods, which involved harsh organic solvents. Second, calcium carbonate can be used as a matrix to trap biomolecules, including proteins and DNA[4], as the processes involved are mild and 'biofriendly'.

The authors began by forming microspheres of calcium carbonate, using them as a matrix to immobilize protein molecules (Fig. 1, overleaf). They also trapped magnetic nanoparticles in the spheres to provide an easy method of separating intermediates from unwanted co-products during the assembly process. The authors coated the spheres with ten alternating layers of synthetic polymers, and then grew a thick shell of calcium carbonate on the surface of the resulting polymer film; a second kind of protein was incorporated into this layer. The authors deposited more layers of polymers onto the outer shell, and finally removed the calcium carbonate by washing with EDTA. This yielded micrometre-sized, shell-within-shell microcapsules, filled with proteins; like a cell, the proteins in the synthetic 'nucleus' were different from those in the 'cytoplasm'.

Both cell and nuclear membranes are semipermeable — they permit some molecules to pass, but not others, which allows many essential reactions to be confined to either the nucleus or the cytoplasm. Although small molecules diffuse through synthetic membranes, many proteins and nanoparticles are retained within LbL capsules, so LbL shells can also selectively control which molecules pass through them.

Kreft and colleagues[1] used this effect to compartmentalize an enzyme reaction. They prepared a shell-within-a-shell system in which enzymes in the outer chamber oxidize water molecules to form hydrogen peroxide. The peroxide readily diffuses into the inner chamber, where it oxidizes an encapsulated dye; this produces a red colour that slowly diffuses back into the outer compartment. This proof-of-principle reaction will no doubt spur interest in other embedded reactions, and perhaps stimulate new designs for synthetic cells.

Compartmentalized structures that mimic cells have been generated using other mild processes. One interesting approach is to trap a solution of two polymers within a lipid vesicle[5]; if the polymers and conditions are carefully chosen, a central pool forms in which