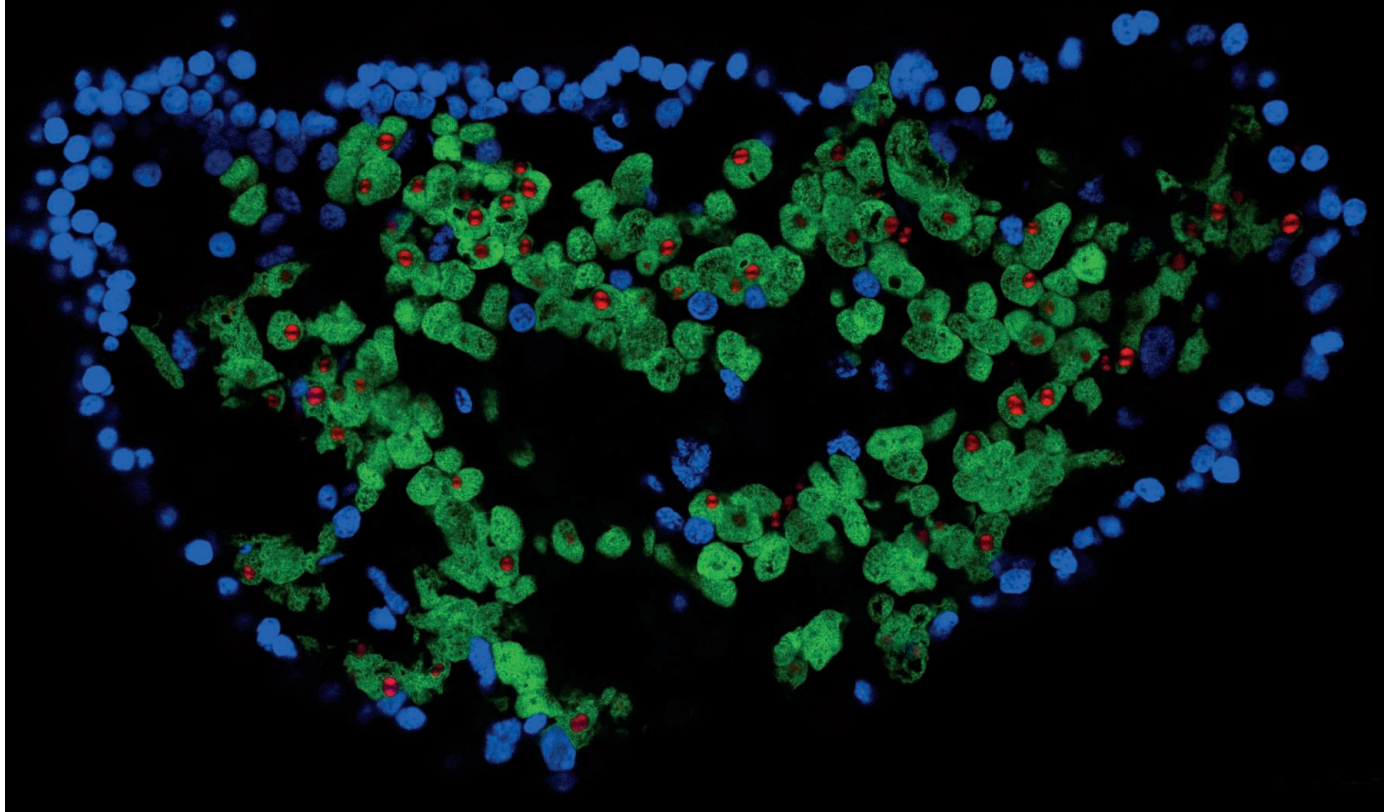


PERSPECTIVES



Mutual dependence. Formerly free-living individuals can become physically, genomically, and metabolically integrated, such as shown in the cross section of *Paracatenula* flatworms colonized by intracellular *Candidatus Riegeria* symbionts (green). Host nuclei are shown in blue and storage compounds in red.

EVOLUTIONARY BIOLOGY

Evolving new organisms via symbiosis

When and how do symbiotic partnerships become new, integrated organisms?

By **E. Toby Kiers¹** and **Stuart A. West²**

Symbiotic partnerships are a major source of evolutionary innovation. They have driven rapid diversification of organisms, allowed hosts to harness new forms of energy, and radically modified Earth's nutrient cycles. The application of next-generation sequencing and advanced microscopic techniques has revealed not only the ubiquity of symbiotic partnerships, but the extent to

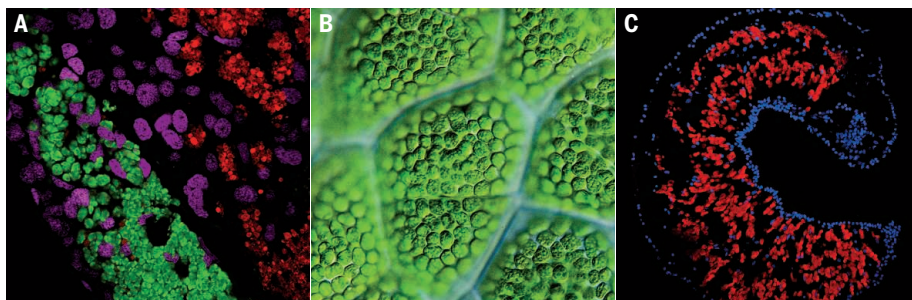
which partnerships can become physically, genomically, and metabolically integrated (1). When and why does this integration of once free-living organisms happen?

Many insects harbor endosymbionts—bacteria that live within the host's cells (see the figure, panel A). Although separate organisms, they function as a metabolic unit. Such dependency can drive extreme genomic integration of host and symbiont at many levels. For example, species of mealy bugs depend on bacterial endo-

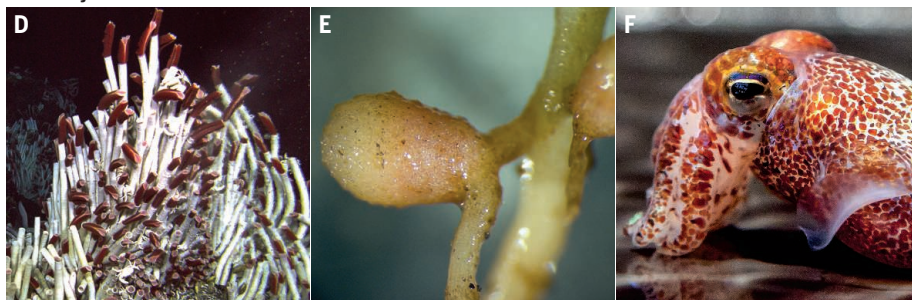
symbionts for nutrient provisioning, and the endosymbiont can in turn harbor its own endosymbiont (2). Patterns of symbiont within symbiont dependencies occur across animals, as well as plant hosts, which use photosynthesizing plastids as a source of energy (see the figure, panel B) (3). Endosymbionts can even speciate within their hosts, as has been found in *Cicada* insects (4).

One potential outcome of host-symbiont integration is a reduction in symbiont

Major transitions



Not major transitions



Major transitions. (A to C) Symbiotic partnership resulting in major transitions in individuality: (A) endosymbionts *Candidatus Hodgkinia* (red) and *Candidatus Sulcia* (green) in a cicada host, with insect cell nuclei shown in magenta; (B) photosynthetic plastids of fern cells; (C) *Paracatenula* flatworm with intracellular *C. Riegeria* symbionts (red) and host nuclei (blue). (D to F) Symbiotic partnership where major transitions in individuality have not taken place: (D) giant marine tubeworm, *Riftia*, which is obligately dependent on nutritional symbionts gained during its larval stage; (E) nodules of legume *Lathyrus japonicus* housing N_2 -fixing rhizobial symbionts; (F) bioluminescent bobtail squid.

genome size. A species of leaf hopper, *Macrostelus quadrilineatus*, harbors the endosymbiont *Nasuia deltocephalinicola*, the smallest bacterial genome sequenced to date (5). Similarities in genome size, coding capacity, and the ability to import proteins produced by the host has driven a debate as to whether such endosymbionts are functionally equivalent to host organelles (6). Organelles evolved when free-living proteobacterial and cyanobacterial ancestors became incorporated into host cells, ultimately forming the mitochondria and plastids that power eukaryotes. Although the debate has largely focused on the genetic and cellular differences between endosymbionts and organelles, it raises a larger evolutionary question: When do we expect symbiotic partnerships to evolve into new, integrated organisms?

Maynard Smith and Szathmáry's major transitions approach provides a framework for addressing this question (7). It focuses on cases where groups of individuals that could previously replicate independently cooperate to form a new, more complex organism. Examples include the formation of chromosomes from the simplest replicators, of the eukaryotic cell from archaeal

and eubacterial cells, and of multicellular organisms through cellular cooperation. By asking what conditions favor these transitions, the framework emphasizes comparisons across different levels of biological organization (8).

The elegance of the major transitions framework is its simplicity. It argues that the same problem—how to overcome the selfish interests of individuals to form mutually dependent cooperative groups—has arisen and been solved at several crucial moments in history across all orders of life. At the same time, it recognizes that transitions in individuality are rare and require strict conditions: Partner interests need to be aligned and the benefits of more integrated cooperation must lead to mutual dependence.

How can group conflict be eliminated and loss of autonomy become favorable? Symbiotic partnerships involve two levels of potential conflict: between symbiont and hosts and among symbionts sharing a host. Hosts have evolved sophisticated mechanisms to manage their microbes, but the resulting symbioses do not necessarily eliminate group conflict (9). Furthermore, although repression of competition is necessary, it is not sufficient to drive a major evolutionary transition, which requires mutual dependence. This can happen when symbionts access new forms of energy for

their hosts, as with mitochondria, and the fitness of partners becomes so intertwined that obligate dependency is favored (10).

Some symbioses are good candidates for major transitions. For example, *Paracatenula* flatworms are colonized by symbionts that harness chemical sources of energy (see the lead photo, page 392). The hosts have evolved such a level of symbiont dependency that they have lost mouths and digestive tracts (see the figure, panel C). In turn, the symbionts have reduced genomes and are passed directly from parent to offspring (vertical transmission) (11). As with some insect endosymbioses (see the figure, panel A), selection has created organism-level adaptations, such as complementary genome modifications, that suggest successful and ongoing major transitions.

To understand when and why new organisms evolve via symbiosis, it is useful to also look at cases where major transitions have not been made, for example when there are asymmetries in dependence. The giant marine tubeworm *Riftia* lacks a digestive system as an adult and depends on a nutritional symbiont gained during the larval stage (12) (see the figure, panel D). Whereas the host has evolved a highly specialized organ to house this symbiont, the bacterial partner retains a free-living stage, is transmitted horizontally, and has not experienced major genome reduction. This suggests that although the partnership provides benefits, there is sufficient conflict or favorable options outside the host to select against symbiont integration.

Other examples where major transitions have not been made include legumes, which are provided with nitrogen by their rhizobia symbionts (see the figure, panel E), and squid, which obtain light for camouflage from bioluminescent bacteria (see the figure, panel F). Both hosts acquire their bacterial symbionts directly from the environment, relying on complex signaling and coordinated molecular pathways to initiate symbiotic development. Yet despite strong coevolutionary histories, partners retain autonomy as individuals (13, 14). The major transitions framework suggests that this is because either strict mutual dependence is not beneficial or there is sufficient conflict between partners or among symbionts. Thus, even when coevolution results in intricate cross-talk and specialized structures to house symbionts, this coordination does not necessarily imply a major transition to a new level of organism.

Major transitions research suggests that the mode of transmission is key to which symbioses form new organisms. When the bacterial partner is acquired directly from the environment, as in the giant tubeworm,

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squid, and legume symbioses, the host is working simultaneously with multiple genotypes. This allows hosts to exploit a wider range of environmental conditions, but also increases the potential for conflict among competing symbionts (15). To help manage conflict, specific structures in hosts (such as crypts in squid) effectively separate different symbiont genotypes into individual chambers (13). In contrast, vertical transmission of symbionts from parent to offspring, as in *Paracatenula* flatworms, can lead to a high relatedness between the symbionts within a host (15), linking the fitness of the symbiont to host performance, reducing conflict, and selecting for integration.

Ecological context can also help to predict when and why transitions have been made (8). When benefits of partnerships vary with environmental context, mutual dependency is less likely to evolve. For example, in the legume-rhizobia symbiosis, the reliance on the bacterial partner decreases in high-nitrogen environments (14). Here, strict dependency can be costly, making a major transition to a single, higher-level organism precarious and unlikely if benefits are not absolute.

The advantage of the major transitions framework is that it emphasizes different questions from the mechanistic ones currently being asked. Interplay between evolutionary theory and genomic research will allow us to understand the evolution of organismal complexity within a single, unified framework. ■

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GEOCHEMISTRY

Biogeochemical tales told by isotope clumps

Molecules with two or more heavy isotopes provide insights into diverse biological and geological phenomena

By Benjamin H. Passey

How do you take a dinosaur's temperature, reconstruct the elevation histories of Earth's great mountain ranges, probe the workings of photosynthesis, and confirm biological origins of a greenhouse gas? Increasingly, the answer lies in clumps. Clumped isotope geochemistry (1) is the latest branch of stable isotope geochemistry, the field that illuminated the Pleistocene glacial-interglacial cycles (2), the oxygenation of Earth's atmosphere some 2.5 billion years ago (3), and the enigmatic presence of grass in hominid diets (4). In this issue, Yeung *et al.* (page 431) (5) and Wang *et al.* (page 428) (6) describe clumped isotope effects that, among other things, can serve as tracers of biological versus abiological origins of gases.

Traditional isotope geochemistry is concerned with ratios of heavy to light isotopes. In contrast, clumped isotope geochemistry looks at occurrences of two or more heavy isotopes in the same molecule, such as $^{18}\text{O}_2$, $^{13}\text{C}^{18}\text{O}^{16}\text{O}$, and $^{13}\text{CH}_3\text{D}$. In its decade of existence, clumped isotope geochemistry has focused on the thermodynamic aspects—particularly the temperatures of molecular and mineral synthesis—recorded in isotopic clumps. Hence, dinosaur body temperatures have been estimated from clumped carbonate ions ($^{13}\text{C}^{18}\text{O}^{16}\text{O}_3^{2-}$) in fossil teeth (7), and altitudes of ancient mountain ranges from $^{13}\text{C}^{18}\text{O}^{16}\text{O}_2$ in fossil soil carbonates (8).

A simple game helps to understand isotopic clumping. Here, we are not interested in the total amount of isotopic clumping, which simply scales with the isotopic composition of the substance. Rather, we seek the deviation from amounts predicted by chance alone. The game is rolling the dice, and the goal is snake eyes—one pip facing up on both dice. The probability of snake eyes for regular six-sided dice is $1/6 \times 1/6 = 1/36$, or 2.77%. The probability changes with the number of sides on each die, becoming $1/4$ for two-sided dice and $1/10^4$ for 100-sided dice. Changing the number of sides is like changing the isotopic composition (9). To win the game, we have to beat chance, and

changing the number of sides will not help.

So far, clumped isotope geochemistry has been concerned with gaming the system on the positive side: coming up with more snake-eyes (or heavy isotope clumps) than predicted by chance alone. This situation is actually preferred by thermodynamics: under conditions of chemical equilibrium, levels of clumping will be slightly higher than levels predicted by chance alone. The isotopic “dice” are weighted by thermodynamics (see the figure). This enrichment in clumps, signified by Δ and on the order of a few parts per thousand, increases as molecular synthesis temperatures decrease (10, 11).

Yeung *et al.* and Wang *et al.* now show that the system can also be gamed in the opposite direction: coming up with fewer snake-eyes

“How can molecules seemingly evade both chance and thermodynamics? The answer appears to relate to the biological assembly of molecules from nonidentical substrate binding sites under irreversible conditions.”

(or fewer heavy isotope clumps) than predicted by thermodynamics and sometimes fewer than predicted by chance statistics. How can molecules seemingly evade both chance and thermodynamics? The answer appears to relate to the biological assembly of molecules from nonidentical substrate binding sites under irreversible conditions.

Photosynthesis generates O_2 by combining oxygen atoms from two water molecules. The oxygen-evolving complex of Photosystem II has two water-binding sites that are thought to be nonidentical. It is therefore plausible that each site has a dif-

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