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Symbiosis: Creating a tractable intracellular insect–microbe association

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Endosymbioses are widespread among insects and have far-reaching implications for their hosts' ecology and evolution. However, the molecular underpinnings of symbiosis remain largely obscure. In a new study, Su *et al.* successfully established a transmissible synthetic symbiosis, opening up exciting new opportunities to explore the initial dynamics of endosymbiotic interactions.

Cereal weevils (*Sitophilus* spp., Coleoptera, Curculionidae) are worldwide pests infesting a large variety of stored grains and cereals. Despite the nutritional limitations of this diet, all stages of the insect exploit the same dietary resource. To cope with this deficiency, the insect is associated with the intracellular endosymbiotic bacterium '*Candidatus* Sodalis pierantonius', which provides its host with vitamins and amino acids[1,2]. In particular, the endosymbiont supports the insect during the early adult phase by producing tyrosine[3], which is a key metabolite for the synthesis, tanning (melanization), and strengthening (sclerotization) of the cuticle. Concordantly, symbiotic beetles exhibit a thicker and darker cuticle in comparison to aposymbiotic (endosymbiont-free) insects. Interestingly, the weevil–*Sodalis* symbiosis is a comparatively recent association[4], and the endosymbiont does not present the typical genome degeneration and reduction that is observed in most other intracellular endosymbionts[2]. As a consequence, this association has been considered a great opportunity to decipher the early steps of endosymbiosis establishment. With its 4.5 Mbp genome and the conservation of numerous metabolic pathways, *S. pierantonius* was envisioned as a promising endosymbiont candidate for culture, which is needed for its genetic manipulation. However, despite these

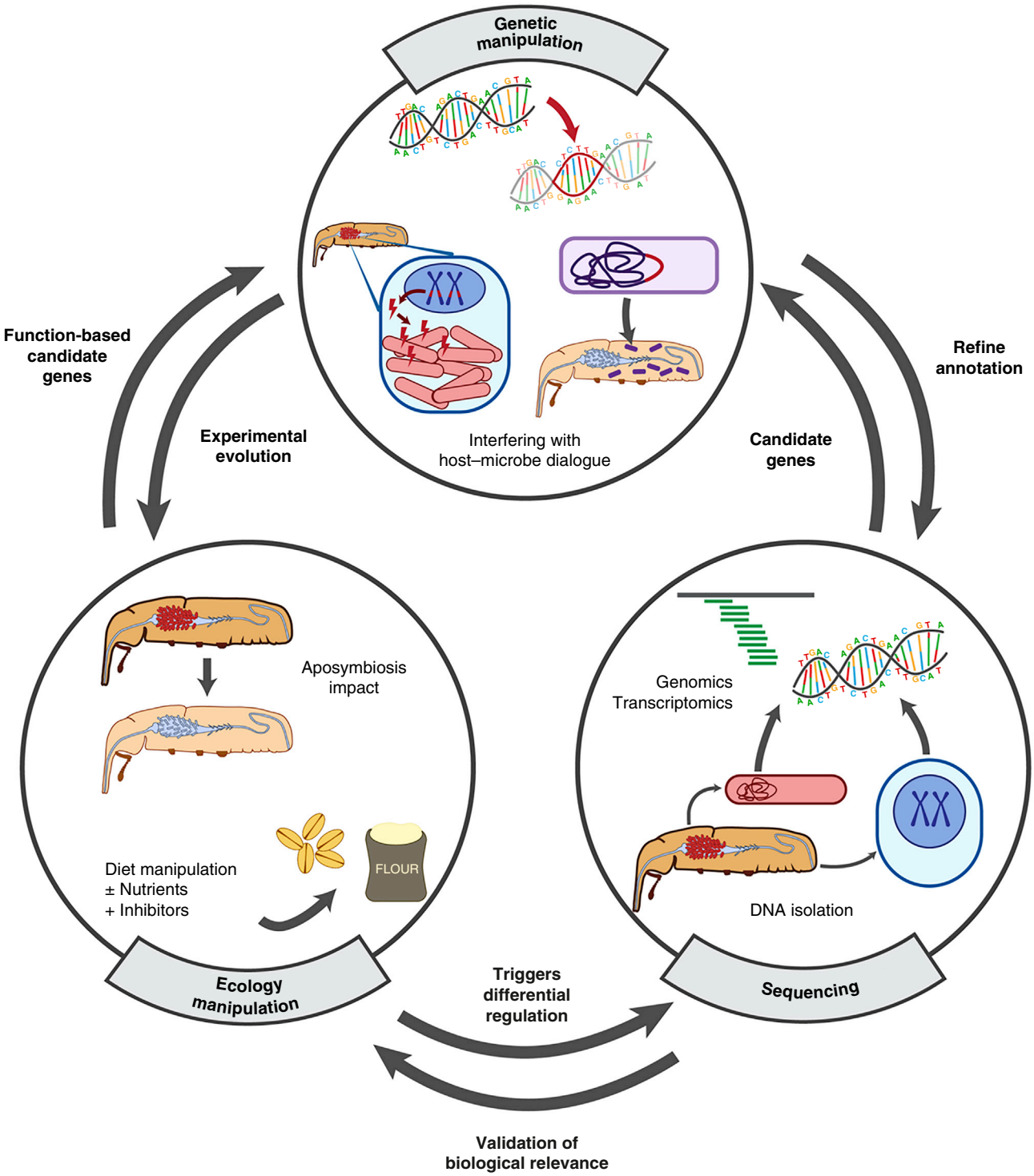

Figure 1. The interconnection of available tools used to investigate endosymbiotic associations.
Understanding the impact of an intracellular symbiont on host biology can be approached by manipulating the environment of the insect (lower left bubble). A common way to assess the importance of a symbiosis is to remove the endosymbiont (by using antibiotics and/or heat shock to generate 'aposymbiotic' insects) and subsequently evaluate the phenotypic impact on the host. Further insights into an endosymbiont's functional relevance can be gained through manipulation of the diet by incorporating or depleting key nutrients. Improved sequencing techniques greatly facilitated the investigation of endosymbiotic associations (lower right bubble). It allows the identification of limited gene sets that are conserved in the reduced symbiont genomes and provides insight into the regulatory pathways involved in the host–microbe dialogue. This increasing availability of sequencing data provides the basis for genetic manipulation (upper bubble),

(legend continued on next page)

promising features, *S. pierantonius* has yet to be successfully cultured.

In contrast to *S. pierantonius*, the close relative *S. glossinidius*, a facultative endosymbiont of tsetse flies, was one of the first endosymbionts to be successfully cultured[5]. However, the *in vitro* cultivation of this bacterium remains tedious and selects for microbes that are poorly transmissible when infected back into their host[6]. The prospect of transfecting bacteria into weevils took a turn for the better with the discovery of *S. praecaptivus*, a free-living, culturable relative of the two insect-associated *Sodalis* species[7]. The injection of *S. praecaptivus* into adult weevils has benign consequences, but the lack of transmission to their offspring limited the use of this bacterium as a tool to explore the mechanisms underlying the beetle's endosymbiotic association[8]. However, this limitation has now been overcome: Su *et al.*[9] report in this issue of *Current Biology* the successful injection of *S. praecaptivus* into weevil eggs, enabling the bacteria to colonize the developing germline of females and subsequently be transmitted to the next generation. Although transmission of *S. praecaptivus* was quite efficient in aposymbiotic insects (78%), the presence of the native endosymbiont undermined the transmission (19%). The mechanisms by which the symbiont is transmitted from the apical bacteriome (the endosymbiont-containing organ) of the ovarioles to the eggs are still unclear, but the presence of both artificial *S. praecaptivus* and native *S. pierantonius* in a single weevil provides an opportunity to study competition for resources and transmission between a native and a newly invading microbe.

Although bacteriocytes, the specialized cells of the bacteriome harboring beneficial endosymbionts, are key in maintaining symbiotic associations across many insects, the mechanisms leading to their formation and differentiation remain obscure. An important observation by Su *et al.*[9] was that newly introduced *S. praecaptivus* colonized prototypical bacteriocytes without inducing their differentiation. This implies that the formation of bacteriocytes relies on the specific interaction between host cells and the native endosymbiont *S. pierantonius*. This is in line with the recent observation that *S. pierantonius* localizes to the bacteriocyte nucleus[10], and further points to a fine-tuned mechanism involving endosymbiont-dependent bacteriocyte development. Further insights into the molecular and cellular pathways structuring and regulating the bacteriocytes have recently been obtained using novel approaches. For instance, the successful *ex situ* culture of another weevil species' bacteriome revealed an intricate dialogue between host and endosymbiont to produce amino acids[11]. Another example is the application of single-cell RNA sequencing to demonstrate differential regulation of bacteriocytes in male and female whiteflies[12]. Single-cell transcriptomics of a developing bacteriome could be particularly useful to discriminate between different stages of bacteriocyte differentiation, and may uncover both host and symbiont genes involved in the process. However, genetic tools have so far been lacking for bacteriocyte-localized symbionts, hampering the functional validation of endosymbionts' candidate genes.

For several extracellular symbioses, genetic manipulation of the symbionts has provided a deeper understanding of the molecular basis underlying insect–microbe interactions[13] and even led to a symbiont-mediated biological control strategy by enhancing insect immune defenses against pathogens[14]. In the new work, Su *et al.*[9] demonstrate the benefit for the insect of being associated with a microbe providing aromatic amino acids. In an elegant set of experiments, the authors engineered *S. praecaptivus* with modified tyrosine and phenylalanine biosynthetic capabilities. Specifically, they generated a tyrosine and phenylalanine auxotrophic strain, as well as a strain overproducing these amino acids. By injecting these strains and the wild type individually into aposymbiotic eggs, they established three different weevil–*S. praecaptivus* associations with divergent interactions: whereas the auxotroph competed with the host for tyrosine and phenylalanine assimilation, the wild-type bacteria were only mildly costly for the host, and the overproducing strain provided tyrosine and phenylalanine to the host. The authors noted that the coloration of the cuticle, which is directly influenced by the amount of phenylalanine and tyrosine available, reflected the nature of the interaction. In addition, weevils associated with tyrosine- and phenylalanine-overproducing bacteria exhibited significantly reduced larval developmental times, indicating that the host derives a benefit from the aromatic amino acid overproducing mutants. Although the outcome of these interacting systems was predictable, they are nonetheless astounding, as it is one of the most direct demonstrations that a change in a single biosynthetic pathway can shift the entire nature of an interaction along the parasitism–mutualism continuum.

In developing their synthetic endosymbiotic system, Su *et al.*[9] have paved the way for an exciting array of future experiments. As they noted, the bacterial genus *Sodalis* seems to be prone to establishing symbiotic associations with insects. In the peculiar case of the cereal weevils, *S. pierantonius* replaced the ancestral weevil endosymbiont *Nardonella*, though the mechanisms by which this replacement occurred remain purely speculative. However, the observation that *S. praecaptivus* co-localizes with the native endosymbiont *S. pierantonius* in bacteriocytes and can be co-transmitted to the offspring, albeit at a low rate, is interesting. The association of the cereal weevils with *S. pierantonius* is considered recent, as evidenced by its large genome and the conservation of virulence factors[2]. The ancestral *S. pierantonius* that first colonized its host may have been functionally close to *S. praecaptivus*

for example, by RNAi or CRISPR/Cas, which can be used in the host insect to knock down, knock out, or knock in candidate genes (red portion of DNA) to validate their function in the endosymbiotic association (red lightning bolts). Editing intracellular symbionts is still limited, but the use of genetically tractable bacteria such as *S. praecaptivus* (purple rounded rectangle) can be a powerful tool in testing or validating the function of bacterial genes in establishing a symbiotic association. Further developing these three approaches — ecological manipulation, sequencing, and genetic manipulation — will push the boundaries of our knowledge on the functional relevance and molecular basis of endosymbiotic associations.

considering their phylogenetic proximity. It is then plausible that what is observed when *S. praecaptivus* infects symbiotic weevils reflects the scenario by which *S. pierantonius* replaced *Nardonella*. Indeed, just as *S. praecaptivus* invades bacteriocytes already housing *S. pierantonius* and transmits by invading the reproductive organs, the ancestral *S. pierantonius* may have also invaded the bacteriocytes and been co-transmitted with, and slowly replaced, *Nardonella* due to better metabolic capacities. Hence, Su *et al.* developed an ideal system to explore potential mechanisms of endosymbiont replacement, or at least the very first steps, by investigating the interaction between a newly invading microbe and an established endosymbiont.

Since the first sequencing of an endosymbiont (*Buchnera*) genome[15], genomic studies have uncovered distinct endosymbiotic features compared to free-living microbes; most notably genome degeneration and reduction in symbionts that have resulted in a minimal gene set, including metabolic pathways beneficial for the host. The growing availability of endosymbiont genomes also provides the basis for genetic modifications aimed at deciphering the functional pathways critically involved in the establishment and maintenance of endosymbiotic associations (Figure 1). However, a substantial limitation remains the unculturability of most endosymbiotic microbes, calling for the development of tools to enable *in situ* editing of endosymbiont genomes. Some promising methods — such as using bacteriophages to specifically target bacterial symbionts — are in development[16]. In the meantime, however, systems such as the artificial weevil–*S. praecaptivus* symbiosis presented by Su *et al.*[9] provide an excellent opportunity to expand our understanding of endosymbiotic associations. For example, experimental evolution can be employed in this system to investigate fundamental aspects of host–symbiont interactions, such as genome erosion, the establishment of efficient vertical transmission, endosymbiont replacement, as well as the regulation of nutritional support. The new work by Su and colleagues is complemented by another recent study from Koga *et al.*[17], who successfully established a novel gut symbiosis in the stinkbug *Plautia stali* with a laboratory strain of *Escherichia coli.* Using experimental evolution and targeted mutagenesis, the authors uncovered that a single mutation in the carbon catabolite repression rendered *E. coli* beneficial to its host, demonstrating that mutualisms can arise rapidly. Despite the difference in nature of the symbioses, both studies highlight the power of engineering experimentally and genetically tractable symbiotic systems, providing opportunities to leverage experimental evolution and track adaptations in real-time. With their work, Su *et al.* made an important step towards establishing the first model for a tractable intracellular symbiosis, which will open a plethora of novel research directions and undoubtedly provide valuable insights into the molecular underpinnings of intimate host–symbiont interactions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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