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Review

The Life of an Insect Endosymbiont from the Cradle to the Grave

John P. McCutcheon[1,4,*], Bret M. Boyd[2,*], and Colin Dale[3,4,*]
[1]Division of Biological Sciences, University of Montana, Missoula, MT, USA
[2]Department of Entomology, University of Georgia, Athens, GA, USA
[3]School of Biological Sciences, University of Utah, Salt Lake City, UT, USA
[4]These authors chose their order based on a coin flip.
*Correspondence: john.mccutcheon@umontana.edu (J.P.M.), bboyd@uga.edu (B.M.B.), colin.dale@utah.edu (C.D.)
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Host-beneficial endosymbioses, which are formed when a microorganism takes up residence inside another cell and provides a fitness advantage to the host, have had a dramatic influence on the evolution of life. These intimate relationships have yielded the mitochondrion and the plastid (chloroplast) — the ancient organelles that in part define eukaryotic life — along with many more recent associations involving a wide variety of hosts and microbial partners. These relationships are often envisioned as stable associations that appear cooperative and persist for extremely long periods of time. But recent evidence suggests that this stable state is often born from turbulent and conflicting origins, and that the apparent stability of many beneficial endosymbiotic relationships — although certainly real in many cases — is not an inevitable outcome of these associations. Here we review how stable endosymbioses form, how they are maintained, and how they sometimes break down and are reborn. We focus on relationships formed by insects and their resident microorganisms because these symbioses have been the focus of significant empirical work over the last two decades. We review these relationships over five life stages: origin, birth, middle age, old age, and death.

Introduction

Over the course of their ~480-million-year evolutionary history [1], insects have repeatedly forged relationships with microbial partners to acquire novel, beneficial functions [2]. These symbioses have often resulted in adaptive radiations for the host insects [3] and have radically altered terrestrial ecosystems [4–6]. The microbial partners provide a wide range of beneficial functions to their hosts, including increased resistance towards stress [7], defense against antagonists [8–11], and even insecticide resistance [12]. However, the most common host-beneficial trait involves the provisioning of nutritional supplements [13–16]. Bacteria and fungi are metabolically diverse, as compared to animals, and can synthesize numerous nutrients that animals (including insects) have lost the ability to make on their own — in particular, the essential amino acids and vitamins. In simple terms, many insects have acquired intracellular microbial symbionts that serve as *live* dietary supplements to facilitate survival on nutritionally incomplete diets.

Here we focus on relationships in which a bacterium (or fungus) lives inside insect cells and is vertically transmitted through host generations. We review these interactions from their beginnings, or births, to their ends, or deaths. We highlight many examples from insects that have associations with *Sodalis*-allied bacterial symbionts, because these symbioses are extremely common and provide insight into the symbiotic process at various time points in our birth-to-death framework. We conclude that although these associations have long been labeled mutualisms—including by us [17,18]—recent data make it increasingly more difficult to see the mutualistic or cooperative aspects of these relationships. We avoid making distinctions between so-called 'primary' and 'secondary' endosymbionts because these terms imply a specific order of symbiont acquisition and ranked importance to the insect host, both of which can change over time and can differ in accordance with host ecological setting. Instead we use the term 'host-beneficial endosymbiont' (HBE) to describe any associate that is known to generate a sustained host benefit.

Origins: Where Do Host-Beneficial Endosymbionts (HBEs) Come From?

Over the past few decades, a great deal of endosymbiosis research has focused on insects, in part because these systems are relatively easy to identify in nature, maintain in the laboratory, and have a small number (one to five) of microbial partners that are often localized to specialized insect tissues at relatively high infection densities. The first efforts aimed at understanding the microbial endosymbionts of insects were based solely on microscopy, and as such the taxonomic origins of the symbionts were difficult or impossible to determine [19]. The application of DNA sequencing to these symbioses immediately revealed the taxonomic classifications of the partner bacteria. For example, even from the first report of the 16S rRNA sequence from the primary bacterial endosymbiont of the pea aphid, *Buchnera aphidicola*, it was clear that this bacterium was a member of the Gammaproteobacteria in the family Enterobacteriaceae [20]. However, such information does not provide much insight into the origin of the symbiont because the amount of family-level sequence diversity in some bacteria is roughly equal to the equivalent amount of sequence diversity in all eukaryotes [21]. Moreover, there is a staggering amount of (mostly unexplored) microbial diversity in the environment that could serve to initiate symbiotic relationships.

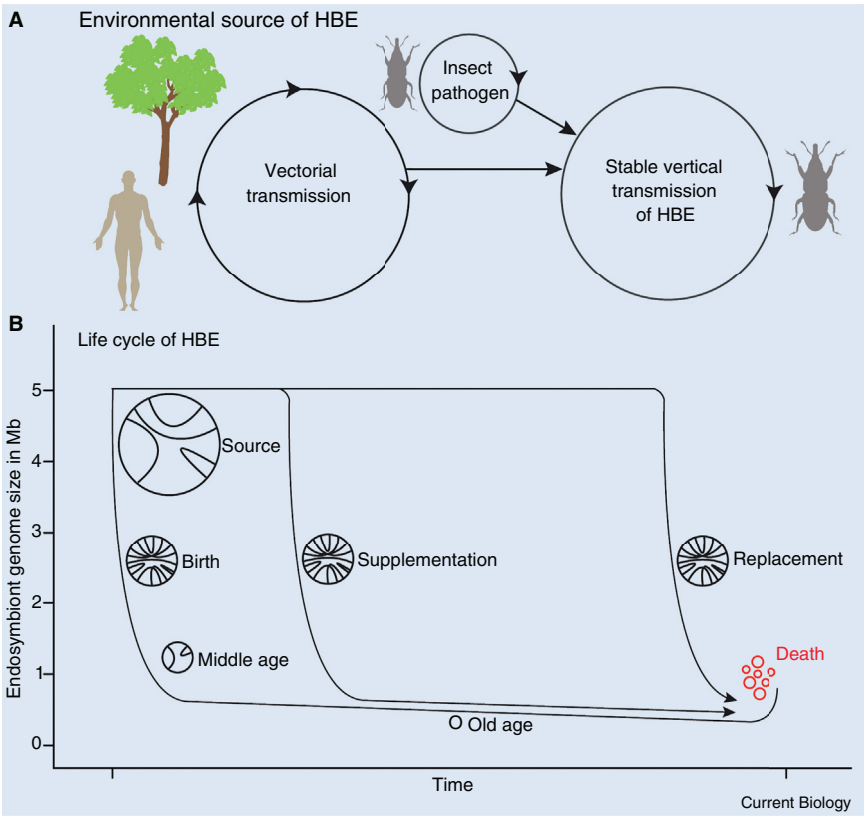

Figure 1. The environmental sources and genomic life cycles of HBEs.
(A) HBEs likely originate from insect-vectored plant and animal pathogens or from direct insect pathogens. The switch to stable vertical transmission by the host locks in the symbiosis. (B) The life cycle of HBE genomes. In all known examples, genome reduction is extremely rapid at the onset of symbiosis and is accompanied by extensive genome rearrangement (shown as inner connecting lines in the genome circles). As the HBE ages, the rate of further gene loss and genome rearrangement slows. Small, stable HBE genomes have been found in numerous insects and can exist for very long periods of time. HBE supplementation can happen, and in most cases the new HBE follows a similar trajectory to the existing HBE. Symbiont death occurs when an HBE is eliminated and replaced by a new organism. Death and replacement may be facilitated in some cases by further symbiont degeneration (shown in red).

HBEs Do Not Often Come from Preexisting HBEs

Interactions between a host and a pathogenic endocellular microorganism are relatively easy to understand. These types of interactions are, by definition, based on conflict. The host is trying to rid itself of the pathogen, and the pathogen needs to circumvent host immune defenses in order to utilize the resources of the host. Because these exploitative interactions inevitably reduce host fitness (sometimes even causing death), pathogenic symbionts must continuously seek to infect new hosts, via a process of horizontal transmission. Sometimes, especially in relatively mild pathogens that form longer-term, chronic infections, the transmission route can be a mix of vertical and horizontal. However, the origin of a pathogenic infection in a new host is typically not mysterious: the simplest hypothesis is that it has arrived horizontally from another host, as a normal part of its life cycle.

In contrast, the origins of HBEs are often much less clear. An HBE that is well adapted to its host has often lost a substantial number of genes compared to free-living relatives [17,22–24]. Comparative genomic analyses indicate that this gene loss takes place rapidly upon transition to a strictly host-confined lifestyle [18], and that the lost genes are dependent on both the host environment and the HBE's role in the symbiosis [25]. In addition, the oldest HBEs, such as mitochondria, chloroplasts, and some endosymbionts in sap-feeding insects, lack many genes that are ubiquitous (and apparently *essential*) in free-living counterparts, implying that they evolved metabolic specificity towards their existing host (through degeneration) and cannot persist outside or easily switch to different—especially distantly related—hosts. Furthermore, many HBEs are transmitted in a strictly vertical manner from mother to egg and have neither the capability nor opportunity to move horizontally. Taken together, these observations suggest that HBEs likely arise independently in host lineages, via capture of an environmental antecedent, and that new associations are unlikely to arise from the horizontal transmission of an existing HBE. One possible exception involves very recently acquired HBEs that have not had sufficient time to evolve host specificity through genome degeneration, but this capability is anticipated to be short-lived as genes are lost and host specificity is enforced by an aggressive process of genome degeneration (discussed below).

HBEs Can Be Recruited from Insect Pathogens

Somewhat paradoxically, a likely source of HBEs may well be pathogens (Figure 1A) [26,27]. Because pathogens often require entry to host cells to complete their life cycles, and because they move between hosts, pathogens have both the ability and opportunity to infect new hosts. If the deleterious impact of infection is mild, it is easy to imagine that a small change in host ecological context could swing the interaction towards a state that is beneficial [26,28]. Evidence for parasitic origins of HBEs comes from systematic studies of endosymbiosis based on statistical analyses of phylogenetic trees [29,30]. Theoretical studies also predict that host-beneficial symbionts should arise from parasites under conditions in which the microbial partner reduces its virulence towards the host and undergoes a switch in transmission strategy from horizontal to vertical [26,31]. Direct, empirical evidence of this pathogen-to-HBE transition comes from fungi in the genus *Ophicordyceps*, which have shown up as beneficial symbionts in numerous insects [31–34]. Recent evidence suggests that the source of fungal HBEs that replaced a bacterial HBE in cicadas was from pathogenic strains of *Ophiocordyceps* fungi [35].

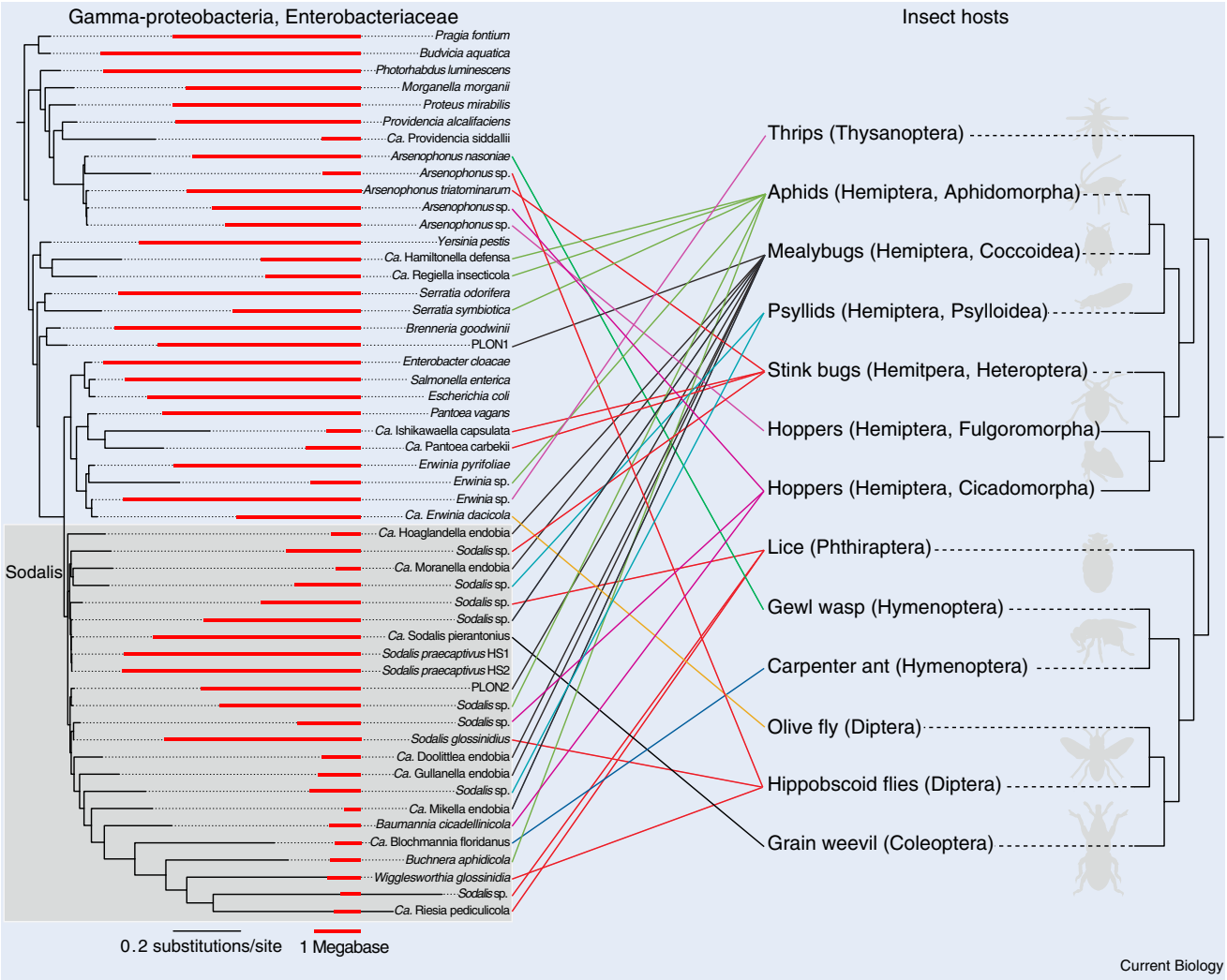

Figure 2. The tangled evolutionary relationships of selected HBEs, their diverse insect hosts, and the range of genome sizes.
Bacterial genome sizes are shown as red bars. The colored lines connect insect groups to known bacterial symbionts. The Enterobacteriaceae phylogeny is based on maximum likelihood analysis of 130 concatenated protein-coding genes from 93 taxa (database construction, tree search, model fitting, and tree refinement methods can be found in the Supplemental Information). The insect phylogeny was adapted from [1]. *Ca*. = *Candidatus*, PLON = *Pseudococcus longipinus* symbiont, sp. = species.

HBEs May Also Come from Vector Interactions

In addition to the notion that HBEs could be derived directly from insect pathogens, it has also been proposed that they might arise from (insect-vectored) environmental plant and animal pathogens (Figure 1A) [36]. In particular, the genome sequences of the *Sodalis*-allied symbionts are all subsets of a recently discovered, closely related environmental antecedent, named *Sodalis praecaptivus* [18]. This bacterium maintains an array of virulence genes that are predicted to assist in the infection of plant, mammalian, and insect hosts. Indeed, *S. praecaptivus* was isolated from a human host who became infected as a consequence of impalement with an infected tree branch [18]. Since *Sodalis*-allied symbionts have also been identified in insects that have longstanding habits of feeding on both plant and animal hosts (Figure 2), it has been postulated that insects may serve as vectors for the transmission of *S. praecaptivus* to plant and animal hosts [18]. This vectorial relationship could promote the formation of a beneficial relationship by imposing a selection pressure that reduces the deleterious impact of the bacterium on its insect vector. Consistent with this idea, *S. praecaptivus* is known to utilize a specialized quorum-sensing system to limit the expression of insecticidal virulence factors to the onset of insect infection, allowing it to maintain a benign and longstanding infection in insects [36]. Furthermore, it is conceivable that a vectored pathogen could evolve host-beneficial trait(s) to offset the negative impact of its maintenance in the vector. It is clear that insect-associated members of not only *Sodalis* but also *Arsenophonus* clades have evolved a host-beneficial symbiotic lifestyle independently on many occasions, over an extensive period of evolutionary time [18]. In the case of *Sodalis,* this is exemplified by the fact that they form a robust phylogenetic clade with a

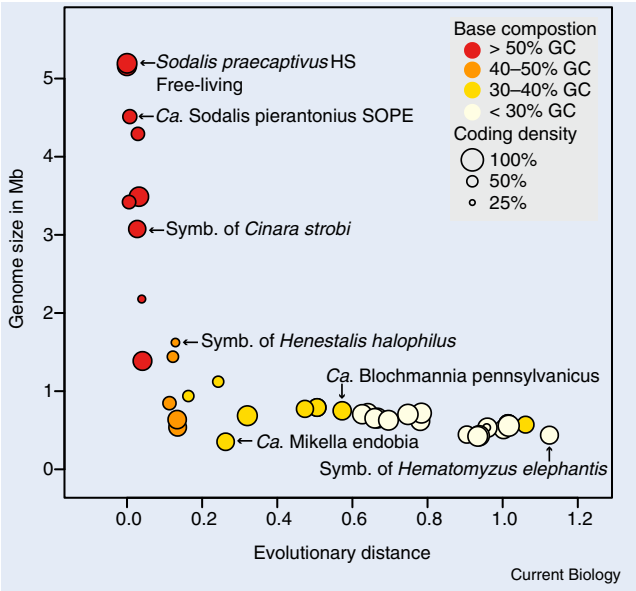

Figure 3. Genome size, sequence divergence, base composition, and coding density for *Sodalis*-related endosymbiotic bacteria at different stages of genome decay.
The initial steep decrease shows that genome reduction is rapid relative to sequence divergence at the onset of symbiosis (schematized in Figure 1B). The trend towards A+T genomic bias can be seen by tracing the dark red points at the top left to the yellow points on the bottom right. Gene density is represented by the size of the points. *Ca*. = *Candidatus*, Symb. = symbiont.

comb-like basal topology, consistent with the notion of independent acquisitions (Figure 2). In addition, the evolutionary distances, or tree branch lengths, between symbiont lineages and free-living relatives are inversely correlated with symbiont genome size (Figure 3).

Microbes Give Up Their Nutrients with Surprising Ease

The derivation of host-beneficial function(s) is of obvious importance in the formation of HBEs. However, free-living microbes often exist in communities in which competition for nutrients is fierce. Provisioning of metabolic resources by symbionts to hosts therefore seems to be in conflict with the basic selfish directive of survival. However, obligate cross-feeding interactions are surprisingly common in microbial communities [37]. In addition, null mutations leading to the loss of function of single genes can substantially enhance the production or release of metabolites such as amino acids by bacteria [38]. The evolution of a host-beneficial genotype is therefore not difficult to rationalize in the case of these insect-microbial symbioses, and once this happens a switch to strict vertical transition can cement the beneficial nature of the association [26]. While little is known currently about the genetic or molecular basis of a transmission switch, it is notable that some insect-infective bacteria are adept at manipulating host reproduction [39,40]. Indeed, a bacterial gene encoding an ankyrin repeat domain-containing protein, which are widespread in the genomes of insect symbionts [41], was recently identified as an etiological agent of male-killing in *Drosophila melanogaster* [42].

Birth: The Traumatic Early Period

By definition, a host that is infected with a pathogenic microorganism incurs a fitness cost. If our hypothesis that HBEs of insects often originate from pathogenic microorganisms is correct, it follows that this fitness cost must be eliminated or significantly reduced when a pathogen makes the switch to becoming an HBE. Evidence suggests that the genomic and functional adaptations that occur during this period are rapid (Figures 1B and 3), but the actual nature of this transition remains poorly understood. In this section we focus on studies that provide insight into this early and mysterious period of HBE evolution, in particular the genomic turmoil that results from this lifestyle transition.

Gaining Access to Host Cells

Gaining access to a host cell is the most rudimentary conflict that is encountered in the evolution of a host–HBE alliance. The host is programmed to treat all invading microbes as foes, and the microbes have to escape host defenses in order to persist. As mentioned previously, pathogenic microbes have the capability to overcome host immune defenses to facilitate infection. Supporting this idea, the gene inventories of recently derived (young) HBEs are found to encode virulence factors that seem to be capable of providing these functions [18]. However, one should not draw conclusions based on sequence gazing alone, because recently-derived HBEs likely maintain many genes that may have played important roles in their former lifestyle but could have no adaptive value in the HBE association. Indeed, one could posit that virulence genes are not at all important to the establishment and function of HBEs because they are not found in the genomes of long-established (ancient) HBEs with highly reduced genomes. Yet, several studies have shown that *Sodalis*-allied HBEs utilize homologs of pathogen virulence genes that either inflict damage upon host tissues in order to facilitate invasion or acquisition of host resources [43,44], or provide resistance towards the killing normally mediated by host innate immunity [44,45]. So why do these virulence genes and functions maintain only a transient existence in the evolution of these associations? This seems to be due to the fact that conflict, which inevitably arises as a consequence of initial infection, is ultimately resolved by synergistic adaptations. For example, the sequestration of bacteria into specialized tissues or cells provides an opportunity to relieve symbionts from the burden of conflict with the host immune system [46], while giving the host the ability to regulate bacterial metabolism by limiting access to certain substrates and facilitating integration of metabolic pathways [47–49].

The Genomic Consequences of the Intracellular Host-Beneficial Lifestyle

The process of genome degeneration that is ubiquitous in insect symbionts and other host-associated microbes has long been predicted to be a consequence of lifestyle constraints afflicting asexual organisms that exist in isolated, clonal populations [50]. These microbes are fated to suffer Muller's ratchet, in which deleterious mutations are fixed at an elevated rate both as a consequence of the relaxed selection imposed by small population bottlenecks encountered during symbiont transmission and because these microbes are asexual and isolated from recombination with compatible bacterial species [50,51]. The ensuing genome degeneration is characterized by a rapid loss of gene

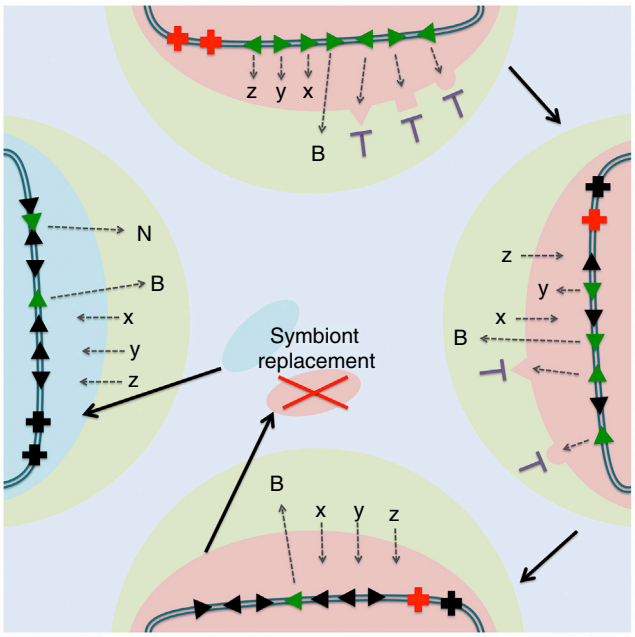

Figure 4. Model describing the impact of mutator phenotypes on HBE evolution.
The evolutionary trajectory of an HBE (pink) is depicted in a clockwise fashion from the top. Degeneration of the HBE gene inventory (dark green triangles) is initially constrained by the requirement to synthesize metabolites (x, y, and z, as well as B, which is uniquely host beneficial) and effect cell wall modifications (depicted as blebs) that are necessary to provide resistance to host immunity. Over time, adaptations in the host cell (light green) facilitate provision of metabolites (x, y and z) and relaxation of immunity, favoring the inactivation/loss of corresponding HBE gene functions (black), which become fixed alongside a mild mutator phenotype in the HBE, depicted by inactivation and loss of a DNA repair gene (red cross). These host adaptations then allow a replacement HBE, one that produces a novel host-beneficial metabolite N, to degenerate in a more rapid fashion, accompanied by a more aggressive mutator phenotype.

functions, arising from a multitude of mutational processes [18,52–54]. While the degenerative trajectory of late HBE evolution can be adequately explained in the context of a reduced efficiency of natural selection [52,54], it is also interesting to note that HBEs often lose components of their DNA replication, repair and recombination systems early in their associations with hosts [55]. Furthermore, in the case of one recently derived *Sodalis*-allied symbiont, the loss of a specific DNA repair pathway has been linked to an increase in gene inactivating mutations resulting from replication slippage events [53]. This loss of repair genes mimics the establishment of mutator phenotypes that often arise in both natural and laboratory-based experimental-evolution populations of bacteria that encounter novel scope for adaptation as a consequence of environmental change or transition towards a more specialized lifestyle [56,57]. Under these circumstances, mutator phenotypes are anticipated to fix by hitchhiking along with the adaptive mutations that they generate.

Locking-In Mutator Phenotypes

If the loss of DNA repair and recombination genes does constitute a transiently adaptive mutator phenotype in HBEs, the most obvious implication is that genome degeneration itself could be adaptive — even in the context of a reduced efficiency of selection — at least in the early stages following lifestyle transition. This is not difficult to rationalize based on the notion that inactivation and loss of genes that encode proteins targeted by the host immune system would benefit symbiont lineages that become less immunogenic. Similarly, increases in fitness may also be associated with the loss of some metabolic genes [58], potentially reducing energy expenditures by the HBE and therefore benefitting its host. Experimental evolution studies also show that deletion of large genomic regions tends to be maladaptive for bacteria, and so mutator phenotypes might speed the generation of strongly beneficial compensatory mutations [54]. Comparative genomic analyses reveal that some ancient HBEs lose >95% of their ancestral gene inventories [17,23], indicating that the transition to endosymbiotic life represents one of nature's most potent opportunities for degenerative genome reduction. However, the establishment of mutator phenotypes does not come without cost [59], nor is it a universal feature of ancient bacterial symbionts [60]. Although such a phenotype might be highly beneficial in the early phase of HBE evolution, when the relative ratio of dispensable-to-essential genes is high, it would be anticipated to become more deleterious over time as that ratio declines. The increased genetic load associated with the mutator phenotype would be expected to exacerbate the effect of Muller's ratchet, potentially reducing the long-term viability of the association. This may have important implications in consideration of the fate of HBEs that are domesticated as first-time associates in completely naïve insects versus those that colonize hosts that have previously harbored HBEs. Bacteria that become established as supplemental or replacement symbionts (detailed in later sections) experience a host environment that is already adapted for HBE maintenance, which may facilitate the establishment of a more aggressive initial mutator phenotype in a newly acquired HBE (Figure 4).

Middle Age: Getting Your House in Order

Following the maelstrom of initial degeneration and adaptation, HBEs tend to settle into a more stable state. They become established residents of specialized host cells, and their genomes tend to become gene dense through the loss of pseudogenes and mobile genetic elements (Figure 1B). In this settled state, it can be useful to think of the HBE gene inventory as an amalgam of 'core' genes that play an essential role in the primary host-benefiting mandate of the symbiosis, and 'housekeeping' genes that have to be retained to ensure that the core mandate can operate. Further gene loss is therefore only possible if the host mandate is reduced in scope, or if HBE gene functions are supplanted by the host [61–63]. Notably, at this stage in the evolutionary process, there is little opportunity, aside from mutational neofunctionalization, to expand the repertoire of HBE functions. As a consequence of genome degeneration, HBEs are 'painted into a metabolic corner' [64].

Getting a Roommate: Symbiont Supplementation

Insect hosts can and do acquire novel HBEs for additional functionality [65] and often these new HBEs evolve in concert with existing HBEs to adopt intricate, interdependent functionalities (Figure 1B) [66,67]. Sometimes, these supplemental

HBE acquisitions occur repeatedly over relatively short evolutionary timescales in the same insect lineage [66,68,69], suggesting that they are more unstable than their primary HBE partners. This is consistent with observations of very high genomic substitution rates [51,70–72] and may be a consequence of more aggressive mutator phenotypes becoming established in HBEs that colonize a pre-adapted host environment (Figure 4). It is also interesting to note that these repeating infections often involve related HBE lineages, perhaps because host adaptations create uniquely favorable conditions for those particular bacterial genotypes or because these bacteria are common enough in the environment to be common sources of HBEs.

The Evolution of A+T Biased Genomes

Another striking and somewhat enigmatic feature of HBEs, possibly related to their mutator phenotypes, involves their almost ubiquitous propensity to undergo an A+T mutational bias (Figure 3) [73]. In one HBE, genomic A+T content has reached 86.5%, leading to a substantial perturbation in the amino acid composition of protein-coding genes and even the loss of a GC-rich codon [74]. The presence of such a bias in HBEs can be explained by aspects of the endosymbiotic lifestyle. First, it is important to note that all bacteria seem to have an A+T mutational bias [75,76]. However, in most cases, the accumulation of A+T is counteracted by a selective force that favors elevated G+C content in coding gene sequences [77] and/or by GC-biased gene conversion (recombination), which favors G+C-rich template DNA [78]. HBEs are thought to evolve under conditions in which the effect of selection is significantly weakened, and so their usual A+T biased genomes are thought to simply reflect the underlying bacterial mutational bias. In addition, many HBEs lack the cellular machinery necessary to perform recombination, and often also lack components of DNA mismatch repair systems that limit the frequency of G+C>A+T substitutions [79]. Although it is difficult to rationalize an adaptive basis for high A+T content in HBE genomes because of the small selective power of individual GC>AT mutations, it is worth noting that the replication slippage events that often catalyze gene-inactivating mutations in the early phase of HBE evolution are known to occur preferentially in G+C-rich DNA [53]. Thus, evolution towards A+T-richness is anticipated to reduce the frequency of these events, stabilizing the HBE gene inventory that emerges from the aggressive early stage of degeneration. Finally, we note that several HBEs deviate from the typical pattern of having A+T-rich genomes and instead have G+C-rich genomes [80,81], in spite of a demonstrable A+T-biased mutation pressure [82]. This implies that in some cases, selection for G+C-rich genomes is strong enough to overcome drift, or that these tiny genomes with high G+C contents undergo host-catalyzed GC-biased gene conversion.

Old Age: Becoming Stable by Managing Conflict

For an endosymbiont, old age is often defined by stability. The chaotic period of establishment is over, and the sorting out of middle age gives way to pronounced genomic stability. The genomes of established beneficial endosymbionts are usually small in size, stable in structure, and dense in gene content. In many respects this is the 'classic' endosymbiotic stage, the one at which most HBEs are observed in nature, probably because this stage can be sustained for extremely long periods of time.

Lessons from Organelle Genomes

The way that we think of organelle genome structure has probably been influenced by the order in which these genomes were reported. Stability in gene content and genome structure was a prominent feature of early mitochondrial [83] and plastid [84] comparisons. The first mitochondrial genomes, mostly from vertebrates, revealed a stable, circular-mapping, 37 gene-encoding, 15–20 kb mitochondrial genome structure that is conserved from humans to trout [83]. But as mitochondrial genomes from more diverse eukaryotes were sequenced, a remarkable amount of structural diversity was discovered. The mitochondrial genome of lice encode the same 37 genes as other animals, but these genes are located on 20 distinct mini-circle molecules [85,86]. Some apicomplexan parasites have tiny six-kb mitochondrial genomes encoding only three genes [87], whereas some plant mitochondria have massively inflated genome sizes (up to 11 Mb [88] in *Silene*) but encode the same genes as most other plant mitochondrial genomes [89]. Excavates such as *Trichomonas vaginalis* have reduced mitochondria that completely lack a genome [90] and some anaerobic microbial eukaryotes have completely lost the mitochondrial organelle (and genome) altogether [91]. The diversity of organelle genome size, structure, and coding capacity is staggering when the breadth of Eukarya is considered, and this diversity suggests that the relative stability of animal mitochondrial genome structure might be more of an outlier than an archetype [92,93].

Genome Stability Is a Common Feature of Ancient HBEs

The field of insect endosymbiont genomics has followed a similar path to organellar genomics. The first two genomes from the aphid endosymbiont *Buchnera aphidicola* showed almost complete structural stability over many tens of millions of years [22,94]. The third *Buchnera* genome was found to be a little different, with a small number of inversions and translocations, but the overall pattern of genome conservation was still strikingly high [95]. Similar genome stability was found in other taxonomically diverse bacterial endosymbionts from cockroaches [96,97], psyllids [98,99], sharpshooters, spittlebugs, cicadas [74], and mealybugs [66]. These results highlight a common feature of HBE evolution during old age: in comparison with their free-living and recently established HBE relatives, long-term HBE genomes converge on a small size, encode few genes, and remain stable in terms of organization and content for tens [94] or hundreds [74] of millions of years.

Genome Stability Likely Reflects Strong Host-Level Selection for Critical Symbiont Functions

If hosts critically rely on endosymbiont function for survival, they can impart very strong purifying selection on them, resulting in stable genomes that change little for long periods of time [100–103]. Does this stability reflect cooperation or conflict? From some perspectives, these endosymbioses might be considered cooperative. The host provides a stable home for the endosymbiont, and the endosymbiont provides a nutritional benefit while allowing the host's adaptive radiation into a new niche that would be inaccessible without the symbiont. The organisms in these associations would therefore appear

to function in a cooperative, mutualistic manner. But from another perspective these relationships might be better thought of as well-managed conflicts, with extreme asymmetrical control, in which the benefit to the endosymbiont is difficult to visualize [28,104–107]. The symbiont certainly gets a huge amount of metabolites, nutrients, and even proteins from its host [49,62,108]. But can this provisioning by the host really be considered a benefit when the symbiont is only reliant on these nutrients because it has lost the ability to make them itself?

Death and Rebirth: Symbionts Burn Out

One way to explore whether these stable endosymbioses are based primarily on cooperation or conflict is to look at what happens when these relationships appear to collapse, perhaps under the weight of a long-term, degradative ratchet of mutations. Cooperation implies two entities working toward a net beneficial outcome, whereas competition implies a power struggle in which each entity is competing for resources. Although evolutionary concepts naturally concern only present interactions and not future outcomes, here we focus on what happens when these intimate associations end. Which of the participating organisms, if any, can persist when one partner is taken away?

Et tu, Mitochondrion?

Of course, some endosymbioses don't seem to end, or at least not on a time scale that we can observe. After all, the eukaryotic mitochondrion has persisted for ~1.8 billion years [109], the plastid for ~1.5 billion years [110], and some insect endosymbionts have been vertically transmitted for greater than 270 million years [111]. These are stable associations by any measure. But recent data show that old endosymbionts are not necessarily permanent [28,112]. Even the mitochondrial organelle, perhaps the ultimate 'cooperator', has now been shown to have been eliminated by one eukaryotic organism: the oxymonad flagellate *Monocercomonoides* has lost not only its mitochondrial genome, but the entire mitochondrial organelle [91]. This loss happened when the organelle's last remaining function, iron-sulfur cluster biogenesis, was made redundant by bacterial genes horizontally transferred to the host genome [91]. Here, the replacing entity was not a new bacterium or fungus, but simply a set of bacterial genes that obviated the need for the host to maintain its minimized mitochondrial function. The host lineage has survived, because it found an alternative solution, but its former mitochondrial lineage has gone extinct.

Insects Can and Do Replace Ancient HBEs

Symbiont extinction and replacement are now well documented in many insect groups [2,105]. Several examples of new bacterial endosymbionts replacing older bacterial symbionts have been reported, including the replacement of one *Sodalis* strain by another numerous times in mealybugs (Figure 2) [66], and a replacement involving *Sodalis* symbionts in louse flies [113]. Bacterial endosymbionts have also been replaced by fungal endosymbionts in many insects, including aphids [114], leafhoppers [115], and cicadas [35]. During the replacement—and especially after—any semblance of cooperation that was involved in the relationship becomes moot. The insect marches on with its new symbiont, but the old symbiont lineage that was replaced goes extinct because it has lost the capability to exist autonomously.

Why Do Extinctions and Replacements Happen?

They might happen because a new endosymbiont supplies some sort of additional beneficial activity that an old endosymbiont was unable to supply (Figure 4). In other cases, replacements might happen just because they can—if the new symbiotic organism is common in the environment constantly infecting insects (Figure 1A), it may replace old endosymbionts by out-competing them for their special intracellular space. But in some cases, it may happen because the old endosymbiont has started to become a liability for the host organism (Figure 1B). Examples of maladaptive symbiont burnout are naturally hard to find because they are transient, but this degeneration–replacement model has been suggested to be occurring in cicadas [35,69,71]. In some cicada species, a single ancestral bacterial lineage has fragmented into numerous degenerate genomic and cellular lineages, which likely arose at a cost to its host insect [116]. This fragmentation-prone endosymbiont has been replaced by a previously pathogenic fungus in at least three cicada groups [35]. It is tempting to speculate that these fungus-bearing cicadas became more fit by domesticating a pathogenic fungus for nutrients than they were when relying on an ancient fragmented bacterial symbiont for those same nutrients [35], but no data on the impact of the replacement have been reported.

Conclusion: The Nihilistic View of Endosymbiosis

We suggest that the labeling of long-term, vertically transmitted endosymbionts that provide a function to a host as willing cooperators, or as partners in mutualisms, is inherently flawed. We find few data that suggest vertically transmitted HBEs benefit in these relationships, apart from (perhaps) a transient benefit at the immediate outset of the relationship when the HBE exploits a novel niche in the environment relative to its free-living counterparts. After this point, the HBE has little influence over its evolutionary fate because it surrenders its capability to adapt to a new environmental niche [105]. In this highly dependent state [117], its fate is then solely determined by the success of a host that can render it expendable by transition into a new environmental niche or by obtaining a novel HBE. We view associations involving HBEs as being built, maintained, and extinguished by conflict-ridden interactions [28] that can nevertheless persist for very long periods of time as a consequence of the functional and adaptive novelty that they generate.

As HBEs age, they tend to lose so many genes that their organismal nature becomes difficult to rationalize. As humans, we romanticize these interactions as highly specialized forms of cooperation because they involve organisms that belong to different domains of life. However, at the level of evolutionary processes, which are purely deterministic, insect–bacterial endosymbioses might be better thought of as adaptive unions of gene functions that happen to originate in distinct cellular genomes. These associations are convoluted, clunky, and conflict-ridden efforts that insects undertake to capture sets of bacterial gene functions that they cannot, for one reason or another, simply capture directly by horizontal gene transfer. Indeed, much of the molecular evolutionary chaos that ensues in the evolution of HBEs is dictated by the idiosyncrasy of

capturing a whole bacterial genome in order to gain just a subset of its functions.

No part of our argument is novel or particularly surprising. The idea that entities at different levels of selection—genes in genomes, cells in organisms, organisms in populations, populations in societies—are in a constant state of selfish struggle is not new [118]. The difference for HBEs, especially those that have been exclusively vertically transmitted for a long time and have lost most of their genes, is that they undergo regression from an autonomous free-living state to a highly-specialized and host-dependent entity that is more akin to an accessory genetic element. In organelles, most [119–121] or in some cases all [91] of the ancestral endosymbiont functions get transferred to the host nucleus. Although there is evidence of horizontal gene transfer in insects that partially replaces HBE function [122–125], this process is not nearly so pervasive as in organelles, perhaps because the complexity of moving all HBE genes to the host nucleus is too high, or because HBEs often lack access to germline nuclei [126], or perhaps just because they are at least a billion years younger.

SUPPLEMENTAL INFORMATION

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