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1 **Convergence of nutritional symbioses in obligate blood-feeders**

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11

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13 **Abstract**

14 Symbiosis with intracellular or gut bacteria is essential for the nutrition of animals with an
15 obligate blood feeding habit. Divergent bacterial lineages have independently evolved
16 functional interactions with obligate blood feeders, but all have converged to an analogous
17 biochemical feature: the provisioning of B vitamins. Although symbionts and blood feeders
18 coevolved interdependence for millions of years, we emphasize that their associations are not
19 necessarily stable. Ancestral symbionts can be replaced by recently acquired bacteria with
20 similar biochemical features. This dynamic emerged through combination of phylogenetic and
21 ecological constraints. Specifically, we highlight the lateral transfer of a streamlined biotin
22 (B₇ vitamin) operon, and conjecture that its extensive spreading across bacterial lineages may
23 drive the emergence of novel nutritional symbioses with blood feeders.

24 **Symbiosis resolved key challenges in obligatory blood diet**

25 **Blood feeding** (See Glossary) is one of the most specialized diets found in animals [1].

26 Blood is nutritionally unbalanced with high levels of protein, iron and salt, but few
27 carbohydrates, lipids and vitamins. Blood feeder genomes evolved large repertoires of genes
28 related to vitamin and lipid shortage, haemoglobin digestion, iron managing or osmotic
29 homeostasis to overcome these dietary challenges [1, 2]. Nevertheless, blood feeders cannot
30 synthesize themselves essential cofactors and vitamins lacking in their diet [3, 4]. Facultative
31 blood feeders, such as mosquitoes and fleas, usually need a blood meal to lay eggs but they
32 also rely on other food sources over their life cycle and then avoid nutritional deficiencies.
33 However, obligate blood feeders (OBF), as ticks, lice, leeches and vampire bats, cannot (**Box**
34 **1**). To overcome this constraint, OBF have converged to analogous functional **microbiomes**
35 with **nutritional symbionts** able to synthesize several **B vitamins**.

36 An obligate blood feeding habit has independently emerged multiple times in animals
37 including insects, arachnids, crustaceans, annelids and mammals, totalizing more than 7,400
38 species (**Box 1**). Accumulating studies demonstrate their ancient associations with B vitamin
39 provisioning symbionts to condition the first appearance of OBF lineages that further radiated
40 into current species [5-10]. The OBF microbiomes are functionally distinct from other
41 animals: invertebrate OBF harbor typical low-complexity microbiomes (e.g. [10-14]), each
42 dominated by one B vitamin provisioning symbiont (**Box 1**), while the only vertebrate OBF,
43 vampire bats, harbor complex gut microbiomes with several potential B vitamin provisioning
44 symbionts but that are distinct to microbiomes of insectivorous, carnivorous and frugivorous
45 bats [15].

46

47 **B vitamin provisioning symbionts as essential partners**

48 Analogous B vitamins-based nutritional interactions appears to be strictly required for the
49 survival and reproduction across the diverse OBF groups. We currently know little about
50 vampire bats, as difficulties to maintain lab colonies make them fastidious experimental
51 models. By contrast, experimental investigations on invertebrate OBF (including ticks, bed
52 bugs, tsetse flies and kissing bugs) showed that, once deprived of their nutritional symbionts,
53 they cease development, stop feeding, molting and reproduction (e.g. [13, 16-18]). They also
54 exhibit physical abnormalities suggestive of a major vitamin deficiency, with dark and
55 inflated bodies. Normal growth and development can be resumed only upon an artificial B
56 vitamins supplementation or symbiont addition. Additional, albeit minor, contributions by
57 nutritional symbionts exist, as exemplified in ticks by the production of the amino-acid L-
58 proline [19].

59 B vitamin provisioning symbionts have evolved narrow associations with their hosts.
60 They colonize only few organs of OBF, mostly **bacteriomes**, where they are hosted
61 intracellularly in symbiotic cells termed **bacteriocytes**, but also gut caecae or **Malpighian**
62 **tubules** in some species (**Figure 1, Box 2**). However, in triatomine bugs, symbionts live
63 extracellularly in the lumen of the gut, although recent investigations also revealed complex
64 microbiomes, including intracellular bacteria that could be additional B vitamin provisioning
65 symbionts. In most cases, B vitamin provisioning symbionts are also heritable through
66 successive generations via high fidelity maternal (usually transovarial, via oocyte infection)
67 transmission (**Figure 1, Box 2**), and are further maintained during the life cycle of their hosts
68 through **transstadial transmission**.

69

70 **The emergence of B vitamin provisioning symbionts**

71 B vitamin provisioning symbionts of OBF originated from at least two bacterial phyla
72 (Proteobacteria and Actinobacteria) and within certain lineages of each phylum, notably the
73 Enterobacteriaceae family (**Box 1 and Table 1**). We conjecture that their emergence in
74 bacterial phyla depends on a combination of phylogenetic and ecological constraints through
75 three mechanisms:

- 76 (i) The first mechanism implies that bacterial ancestors were already adapted to early
77 mutualistic nutritional lifestyles, but with non-OBF organisms. Indeed, many
78 Enterobacteriaceae are facultative symbionts, *ie*, non-required for host survival,
79 but they are well known to influence animal nutrition and metabolism in diverse
80 ways, and many are nutritional symbionts of non-OBF organisms. As such, some
81 are extracellular symbionts inhabiting gut of vertebrates, others are intracellular
82 symbionts of diverse arthropods, and most can produce B vitamins [20]. The
83 combination of their broad distribution in animals and their biosynthesis capacity
84 makes them a breeding ground for evolving nutritional symbiosis with OBF: the
85 emergence of *Wigglesworthia* symbiont in tsetse flies, *Riesia* in lice and
86 *Providencia* in leeches appears to occur through maintenance of ancestral B
87 vitamin genes in these Enterobacteriaceae groups [6, 21, 22].
- 88 (ii) The second mechanism implies that bacterial ancestors were not adapted to a
89 mutualistic nutritional lifestyle, but to a parasitic lifestyle with non-OBF
90 organisms. This mechanism relies on maintenance of ancestral bacterial genes that
91 encode for **pre-adaptations** to nutritional symbiosis, but that were primarily
92 dedicated to another function, as best exemplified with the γ -proteobacterium

93 *Francisella* associated with ticks. It has emerged from a clade of virulent
94 intracellular pathogens of vertebrates that includes the agent of tularemia *F.*
95 *tularensis* [17, 23]. *Francisella* pathogens have evolved specific mechanisms to
96 penetrate into phagocytes of mammals, and the self-production of biotin (B₇
97 vitamin) is here a key factor that enables pathogen replication and ultimate escape
98 from the phagosomes [24, 25]. In the *Francisella* genus, the biotin biosynthesis
99 pathway has evolved in the context of pathogenesis before being coopted for
100 nutritional symbiosis in ticks.

101 (iii) The third mechanism depends on **lateral gene transfers**. Several B vitamin
102 provisioning symbioses have independently evolved following biotin gene uptakes
103 in the Rickettsiales order (Alpha-proteobacteria): *Wolbachia wCle* in bed bugs,
104 *Midichloria* in the castor bean tick *Ixodes ricinus* and *Rickettsia buchneri* in the
105 black legged tick *I. scapularis* (**Figure 2**). All Rickettsiales are intracellular: some
106 are pathogens, such as the agent of epidemic typhus *Rickettsia prowazekii*, while
107 others are **reproductive parasites** of arthropods, such as *Wolbachia*, but only few
108 harbour B vitamin genes [26-28]. Phylogenomic reconstructions revealed that
109 three independent acquisitions of a streamlined biotin operon are at the origin of
110 the Rickettsiales nutritional symbioses currently found in bed bugs and ticks [26-
111 28]. While the acquisition of special 'symbiosis' genes is usually rare for
112 nutritional symbionts [4, 29], the Rickettsiales nutritional symbioses show that
113 foreign gene uptakes are key drivers of interactions with OBF. However, a
114 different mechanism operates for folate (B₉): the folate biosynthesis pathway was
115 early present in the Rickettsiales ancestor but secondarily lost in most sub-lineages

116 [30]. Remarkably, all the folate biosynthesis genes have been consistently
117 maintained in Rickettsiales symbionts of bed bugs and ticks [26-28].

118

119 **Convergence of B vitamin provisioning symbioses**

120 Bacteria adapted to symbiotic (and specifically intracellular) lifestyles underwent massive
121 genome reduction (**Box 3**). The gene set of B vitamin provisioning symbionts is largely a
122 subset of the gene repertoires of their relatives: non-necessary genes have been pseudogenized
123 or are missing completely, but B vitamin synthesis pathways have been conserved [6, 13, 17,
124 21, 22, 31-33]. However, depending on OBF symbiotic systems, certain B vitamin synthesis
125 pathways have been maintained intact while others have been degraded or lost (**Table 1**).
126 Notably, the symbiont genomes consistently harbour biosynthesis pathways of biotin and, at
127 lesser extent, folate and riboflavin (B₂): these three pathways form a set of core genes fitting
128 with the nutritional need of OBF. Each of the B vitamins are required for key enzymatic
129 reactions in animals: biotin is a coenzyme for carboxylase enzymes, needed for fatty acids
130 synthesis, branched-chain amino acid catabolism, and gluconeogenesis; folate is a precursor
131 essential for the synthesis of DNA, the modification of DNA and RNA, and is also an
132 important cofactor for cellular metabolism; riboflavin is a precursor of flavin mononucleotide
133 (FMN) and flavin adenine dinucleotide (FAD) coenzymes, which are needed for a variety of
134 flavoprotein enzyme reactions, including activation of other vitamins.

135 The presence of other B vitamin genes is more variable, and some pathways are missing
136 one or more genes, or are entirely absent (**Table 1**). This pattern may actually depend on the
137 symbiont lifestyle and the specific nutritional need of certain OBF. Indeed, the symbiont
138 *Rhodococcus rhodnii* of kissing bugs has complete gene sets for the eight B vitamins (**Table**

139 1), suggesting that all are potentially needed either for kissing bug life cycle, or for *R. rhodnii*
140 growth, or both. As an extracellular gut symbiont, *R. rhodnii* is exposed to fluctuating
141 environments, in and out of host (it is transmitted by feces; **Figure 1**). This lifestyle is
142 reflected in its large genome (4.3Mb) which exhibits important gene clusters dedicated to
143 antimicrobial molecules and metabolic plasticity, and the eight B vitamin pathways may
144 contribute to this plasticity [34]. By comparison, most B vitamin provisioning symbionts of
145 OBF are intracellular; they live in a more stable, predictable and protected environment. As
146 such, their metabolic needs are more limited and they have small genomes, lacking genes in
147 almost all functional categories [29, 35, 36]. Hence, most intracellular symbionts have
148 maintained intact the pathways for only few B vitamins other than biotin, ribflavin and folate
149 (**Table 1**). However, some intracellular symbionts, as *Wigglesworthia* symbiont in tsetse flies
150 and *Riesia* in lice, can produce most B vitamins (**Table 1**), suggesting that their hosts need
151 this provisioning to their own growth and reproduction.

152

153 **The fragile stability of B vitamin provisioning symbioses**

154 B vitamin provisioning symbionts and OBF evolve a narrow interdependence, and each
155 cannot survive without the other. Thanks to this **coevolution**, OBF nutritional symbioses have
156 been traditionally envisioned as stable associations lasting for millions of years and resulting
157 in **co-cladogenesis**, as shown in tsetse flies, and certain clades or genera of bat flies, louse
158 flies, ticks and lice [5, 7-9, 13]. However, recent observations reveal that OBF nutritional
159 symbioses are much more dynamic. Notably, nutritional symbioses can break down: recently
160 acquired symbionts can replace ancestral B vitamin provisioning symbionts and provide
161 similar benefits to the host. Such a pattern was observed in louse flies with the recent

162 acquisition of a *Sodalis* symbiont [38]. In sucking lice, there are up to six independent
163 lineages of B vitamin provisioning symbionts [6, 7, 39-43], which suggests recent origin of
164 each lineage and therefore several replacement events. In ticks, there are at least four
165 independent lineages of B vitamin provisioning symbionts (*Coxiella*, *Francisella*, *Rickettsia*
166 and *Mitochondria*) [9, 16-17, 19, 23, 26, 28, 31, 33]. Notably, *Coxiella* symbioses are ancestral
167 in some tick genera but recent replacements by *Francisella* in some species appear across the
168 tick phylogeny [5, 9]. Genome sequencing otherwise confirmed that *Coxiella* and *Francisella*
169 have roughly similar B vitamin biosynthesis capabilities: the recently acquired *Francisella*
170 provides the same B vitamin benefit to ticks as the ancestral *Coxiella* (**Table 1**) [17, 19, 23,
171 31, 33].

172 Why ancestral and co-evolved nutritional symbionts are replaced in OBF remains
173 unresolved, but several mechanisms can be proposed. Indeed, ancient symbionts suffer
174 Muller's ratchet, with fixation of deleterious mutations through genetic drift, and they may
175 just have over degraded genomes (**Box 3**). In this context, the comparison of OBF with sap-
176 feeding insects is instructive: they host symbionts compensating for nutritional deficiencies of
177 the sap diet. In sap-feeders, the most severely reduced of symbiont genomes are missing
178 genes usually considered to be essential and harbor the tiniest known bacterial genomes.
179 However, these nutritional symbioses do not collapse thanks to diverse mechanisms recently
180 observed, including evolution of novel traits by hosts to compensate for symbiont gene losses,
181 acquisition of another symbiont to supplement (or replace) functions that are lost in the older
182 symbiont, or DNA uptake from environmental microbes to replace lost symbiont genes. These
183 mechanisms are all potentially applicable to the symbioses with OBF. In ticks, such a pattern
184 was observed for *Coxiella* with loss of essential genes for their replication, offering the

185 opportunity to another member of the microbiome to out-competing them [31, 33]. In
186 addition, recently acquired symbionts may have higher biosynthetic capability than ancestral
187 symbionts, and then supply additional benefits to OBF. In ticks, some ancestral *Coxiella* have
188 genomes of only 0.66 Mb [33] while the recently acquired *Francisella* have bigger genomes
189 (>1.5 Mb) that may have higher biosynthetic capability [17, 23]. This degeneration–
190 replacement model has been proposed for other nutritional symbionts of arthropods [44-46],
191 but are difficult to observe since replacements are expected to be transient [29]. However, the
192 recent observation of a few tick species with co-infections by ancestral *Coxiella* and recently
193 acquired *Francisella* may correspond to this transient state before extinction of the ancestral
194 symbiont [5].

195

196 **Invasion of a streamlined biotin operon**

197 Accumulating genomic sequences confirmed that lateral transfer of a compact,
198 streamlined, biotin operon is rampant in OBF nutritional symbioses: related biotin operons
199 (i.e., that diverged recently from the same operon ancestor) were detected in diverse B
200 vitamin provisioning symbionts of OBF (**Figure 2**, [26, 27, 43]). Related operons were also
201 found in other intracellular bacteria, mostly in symbionts of non-OBF arthropods, such as the
202 **reproductive parasite** *Cardinium* [47]. The incongruence between bacterial and operon
203 phylogenetic trees underlines that these streamlined biotin operons experience recent (and
204 likely ongoing) transfers between distantly related bacterial lineages (**Figure 2**). Its invasive
205 nature may have contributed to major evolutionary innovations through the emergence of
206 novel OBF nutritional symbioses. What is yet to be established are the mechanisms
207 underlying the invasive nature of this streamlined biotin operon above other biotin operons.

208 These mechanisms may operate on different levels: the primary acquisition of the operon
209 depends on the opportunity to DNA uptake from other bacteria, while its success to spread in
210 the symbiont population rather depends on selection acting on the benefit it provides.

211 Lateral gene transfer is usually thought to be rare in intracellular symbionts as they reside
212 in confined and isolated environments. However, according to the ‘intracellular arena’
213 hypothesis [48, 49], coinfections of different symbionts within the same host cell, and the
214 propensity of some symbionts to switch between arthropod hosts, have created freely
215 recombining intracellular bacterial communities [48-51]. The detection of the streamlined
216 biotin operon in *Wolbachia*, *Rickettsia* and *Cardinium* [26, 27, 47] that are three of the most
217 common intracellular symbionts of arthropods [52, 53], corroborates the ‘intracellular arena’
218 hypothesis and its role in emergence in novel OBF nutritional symbioses.

219 Once acquired, the further maintenance and spread of the operon in the symbiont
220 population may be indicative of positive selection acting on it. A possibility is that the
221 streamlined biotin operon is more efficient in producing biotin than others. In other operon
222 systems, there are selective pressures for efficient specific gene orders and reduced intergenic
223 regions to optimize the expression and functionality of operon in general [54, 55]. Others
224 mechanisms may include the streamlined nature of this operon itself: its compact gene
225 structure may favor its transfer in a single genetic block to other bacteria. Alternatively, the
226 streamlined biotin operon may have genomic features favoring its transposition, but such a
227 mechanism has not been detected to date.

228

229 **Concluding remarks**

230 That feeding specialization to strict blood diet is driven by nutritional symbioses is now
231 beyond doubt. The capacity to synthesize B vitamins is widespread in bacteria but the
232 nutritional symbionts of OBF have all converged to analogous, critical, interactions with their
233 respective hosts. This convergence consists in severe degeneration of bacterial genomes
234 accompanied by preservation of some B vitamin biosynthesis pathways, or in some cases, by
235 a secondary acquisition of the streamlined biotin operon. The OBF nutritional symbioses can
236 be ancient and highly co-evolved. However, we have now to consider that they are also
237 influenced by a dynamic and complex web of interactions by which symbionts move between
238 hosts and genes move between symbionts. We postulate that a better characterization of this
239 web of interactions is now required if we are to understand the mechanisms driving the
240 different aspects of nutritional symbiosis with OBF, such as convergence of B vitamin
241 biosynthesis capacity, instability of association, extinction of ancestral symbiont, acquisition
242 of novel symbionts or invasive spread of the streamlined biotin operon (see Outstanding
243 Questions). Ecological opportunities, along with phylogenetic constraints and selective
244 pressures acting on symbiotic systems, may altogether explain common and divergent
245 evolutionary patterns in OBF nutritional symbiosis. In this context, comparative ecological
246 and genomic approaches will be highly valuable in enhancing understanding of OBF
247 nutritional specialty via symbioses.

248

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256

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394

395 **Glossary**

396 **Bacteriocyte:** Specialized giant cell (also known as mycetocytes) of certain insects as tsetse
397 flies, bat flies, bed bugs but also aphids or weevils. It specifically contains endosymbionts
398 which provide essential amino acids and other molecules to their hosts, including B vitamins.

399 **Bacteriome:** Microbes containing cell clusters and surrounding cells which form altogether a
400 symbiotic organ. Depending on species, bacteriomes are found near the gut, or in the gut wall,
401 the gonads or nested among other organs within abdominal body cavity.

402 **Blood feeding:** The practice, either occasional or exclusive, of certain animals to feed on
403 blood of vertebrates. Also known as blood sucking, hematophagy or sanguinivory.

404 **B vitamins:** A group of chemically diverse water-soluble vitamins, commonly synthesized by
405 microorganisms, that are essential for cell metabolism and cannot be produced by animals.

406 The eight types of B vitamins are: thiamine (B₁), riboflavin (B₂), nicotinic acid, also known as

407 niacin (B₃), pantothenic acid (B₅), pyridoxine (B₆), biotin (B₇), folate (B₉) and cobalamin
408 (B₁₂).

409 **Co-cladogenesis:** The parallel process of speciation between host and symbiont, such that
410 phylogenetic trees of each partner are congruent.

411 **Coevolution:** The evolution of two or more species which reciprocally affect each other
412 through the process of natural selection.

413 **Lateral gene transfer (LGT):** The movement of genetic material (including genes, operon,
414 genomic islands, or more broad genomic regions) between unrelated organisms that may
415 belong to different genera, families, orders, classes, phyla or even kingdoms, and that usually
416 do not broadly exchange their genetic material. Also known as Horizontal gene transfer
417 (HGT).

418 **Malpighian tubules:** Excretory and osmoregulatory organs of arthropods that lie in the
419 abdominal body cavity and empty into the junction between midgut and hindgut.

420 **Microbiome:** The community of commensal, mutualistic and pathogenic microorganisms that
421 reside in an environmental niche (such as an animal for instance).

422 **Nutritional symbionts:** microbes, usually bacteria and yeasts, that synthesize key nutrients
423 lacking in the hosts' diet.

424 **Pre-adaptations:** The possibility of a certain characteristic trait to adopt a new biological
425 function without evolutionary modification, or to have the same function in a different
426 environmental context.

427 **Reproductive parasite:** Maternally inherited microorganisms that spread within arthropod
428 populations by manipulating the host reproductive processes to enhance their own
429 transmission. Manipulations involve biasing the sex ratio of infected females towards the

430 production of daughters through parthenogenesis, feminization of genetic males or male
431 killing. Some reproductive parasites also induce reproductive incompatibility (cytoplasmic
432 incompatibility) between males infected with a particular strain of bacteria and females not
433 infected with this strain.

434 **Transovarial transmission:** Transmission via oocyte infection.

435 **Transtadial transmission:** Transmission during ontogeny from one life cycle stage to the
436 next.

437 **Box 1. Diversity of obligate blood feeders and their symbionts**

438 Obligate blood feeding (OBF) habit has independently emerged multiple times in animals.

439 There are more than 7,400 OBF species currently known worldwide [60]. Most of the OBF
440 species are found in insects including:

441 • Sucking lice (Anoplura), with more than 5000 species. The Anoplura are all blood-feeding
442 ectoparasites of mammals. A few species are parasites of humans, including the human body
443 louse *Pediculus humanus humanus*, and the human pubic louse *Phthirus pubis*. Body lice are
444 vectors for the transmission of the human diseases: epidemic typhus, trench fever, and
445 relapsing fever.

446 • True bugs (Heteroptera), with at least 100 species of cimicids (bed bugs and relatives), 32
447 species of Polyctenidae bat bugs and more than 130 species of kissing (aka assassin,
448 triatomine or vampire) bugs. Most species of cimicid are specialised on insectivorous bats or
449 birds, while a few species, the common bed bug *Cimex lectularius* and its tropical relative *C.*
450 *hemipterus*, feed on humans. Kissing bugs share shelter with nesting arboreal vertebrates,
451 from which they suck blood. They are mainly found and widespread in the Americas where
452 they are vectors of the Chagas disease parasite *Trypanosoma cruzi*.

453 • True flies (Diptera) with tsetse flies (>20 species), bat flies (>500 species) and louse flies
454 (or keds, >150 species). They belong to the superfamily Hippoboscoidea and are obligate
455 parasites of mammals and birds, often with a crab-like (louse fly) or a spider-like (bat fly)
456 appearance. They reproduce through adenotrophic viviparity (see **Box 2**). Tsetse flies are
457 vectors of trypanosomes, which cause human sleeping sickness and animal trypanosomiasis.

458 Other OBF species exist in arachnids:

459 ● Ticks (Acari: Ixodidea), with more than 900 species. Depending on species, they feed on
460 mammals, birds, but also reptiles and amphibians. Best-known tick-borne disease is the Lyme
461 disease that is nowadays a widespread infectious disease in the northern hemisphere.

462 ● Other Acari groups, with an undetermined number of species, such as the poultry red mite
463 *Dermanyssus gallinae* (vector of avian spirochaetosis), and the snake mite *Ophionyssus*
464 *natricis* (vector of ophidian paramyxovirus).

465 Only few other OBF species exist in non-arthropod groups:

466 ● Leeches (Hirudinea), with more than 700 species. The salivary glands of these annelids
467 produce an anticoagulant peptide, hirudin, used to treat some blood-clotting disorders.

468 ● Fish lice (crustacean subclass Branchiura), with approximately 120 known species. They
469 are the most widespread ectoparasites of freshwater fish in the world and can cause the severe
470 disease state argulosis. All life stages of both sexes are parasitic.

471 ● Vampire bats (Chiroptera: Desmodontinae), with only three species. All are native to the
472 Americas. Although rare, vampire bat bites can infect humans by rabies.

473 Phylogeny of representative B vitamin provisioning symbionts associated with these obligate
474 blood feeders is depicted in Figure I.

475

476 **Figure I. Evolutionary relationships of major examples for B vitamin provisioning**
477 **symbionts associated with obligate blood feeders.** The phylogeny is based on widely
478 supported findings from studies listed in the citations. B vitamin provisioning symbionts
479 associated with obligate blood feeders are shown in red. The bacterial phylogeny was drawn
480 from iTOL (<https://itol.embl.de/>).

482 **Box 2. Localization and transmission of B vitamin provisioning symbionts**

483 Reliable transmission mechanisms of B vitamin provisioning symbionts are necessary to
484 stabilize nutritional associations between generations of OBF, and, more broadly, over
485 evolutionary time. The efficiency of symbiont transmission can be enhanced by the evolution
486 of co-adapted traits that ultimately lead to greater interdependence. Each lineage of OBF
487 exhibits specific traits that condition what mechanisms of symbiont transmission is more
488 favorable. In most OBF harboring **bacteriocytes**, such as bed bugs and lice, B vitamin
489 provisioning symbionts are transmitted vertically through transfer from the maternal
490 bacteriocyte to the ovaries of the female host, and thence to the eggs, a process known as
491 **transovarial transmission** (see also **Figure 1**). In other obligate blood feeders harboring
492 bacteriocytes, such as tsetse flies, bat flies and louse flies, the transmission of B vitamin
493 provisioning symbionts arise latter during the insect development. Females of tsetse flies, bat
494 flies and louse flies retain each egg within her uterus to have the offspring develop internally
495 during the first three larval stages, a method called adenotrophic viviparity. During this time,
496 the female feeds the developing offspring with a milky substance secreted by a modified
497 gland in the uterus. The milky substance contains B vitamin provisioning symbionts which
498 are then transmitted to the developing insect larvae. In triatomine bugs, the B vitamin
499 provisioning symbionts show an exception among invertebrates as they are found in the gut,
500 free of host cells and are transmitted to offspring via the feces either by egg shell
501 contamination or by coprophagy, or even via cannibalism. The excretion of large quantities of
502 water following the quick processing of the enormous volume of ingested blood, combine
503 with the gregarious behavior of the triatomines, may have facilitated the evolution of this
504 transmission mode. In vampire bats, we currently do not know how individuals acquire their

505 B vitamin provisioning symbionts, but it seems probable that transmission takes place at the
506 time of birth when a newborn is exposed to a mother's microbiota.

507 **Box 3. Genomic decay in bacterial symbionts**

508 Nutritional symbionts required for host survival harbor unusual genome modifications,
509 including extreme reduction, rapid protein evolution, low GC-content and codon
510 reassignments [4, 29, 36]. The process of genome reduction is initiated as a consequence of
511 loss of selection on multiple gene functions when a free-living bacterium becomes host-
512 associated [61]. The within-host environment, particularly inside a host cell, is relatively
513 stable and nutrient rich, and the need for motility, regulation, secondary metabolite
514 biosynthesis and defense is largely lost: over time the genes encoding such functions are
515 pseudogenized and further definitely lost [4, 29]. For nutritional symbionts required for host
516 survival, this process can continue even further, leading to tiny genomes where even genes
517 considered essential are lost [4, 29, 36]. Symbiont genome decay however affects genes in all
518 functional categories, even those involved in beneficial interactions with hosts [4, 29, 36].
519 Symbiont genomes continuously accumulate deleterious mutations and their degeneration
520 may ultimately lead to maladaptation and then limit their beneficial contributions to their
521 hosts. A main driving force for this process comes from strict clonality and small population
522 size of nutritional symbionts during transmission, on which Muller's ratchet effect, leading to
523 elevated rates of fixation of deleterious mutations irreversibly. Genetic drift favors the
524 fixation of neutral or deleterious mutations that cause gene inactivation, gene loss, or
525 inefficiency of gene products. Indeed, gene products of nutritional symbionts have lower
526 efficiencies and reduced thermal stability than their homologs in free-living relatives. Once a
527 bacterium has proceeded down the irreversible path into such obligate symbiosis, there is little
528 opportunity to exit and the nutritional symbiosis can break down and collapse [29, 36].

529 **Figure 1. Major examples for tissue tropism and mode of inheritance B vitamin**
530 **provisioning symbionts associated with obligate blood feeders.** Red dots: B vitamin
531 provisioning symbionts in major hosting organ; Violet dots: B vitamin provisioning
532 symbionts in gonads (for maternal transmission, excluding kissing bugs); Violet organs:
533 gonads/milk glands (louse flies); Yellow organs: midgut/Malpighian tubules (ticks). EB-
534 esophagus bacteriome; MGT- midgut; MGL-milk glands; MT-Malpighian tubules; OA-ovary
535 ampule; OB- ovarian bacteriome; Ov- ovaries; SD- stomach disk; TB- testis bacteriome.
536 Drawings are based on representative work of each taxon: Lice [32, 40]; ticks [17, 56];
537 Bedbugs [13]; Louse flies [57]; kissing bugs [58]; leeches [22, 59].

538

539 **Figure 2. Evolutionary relationships, origin and structure of the streamlined biotin**
540 **operon.** The figure is based on widely supported findings from previous studies. Red, B
541 vitamin provisioning symbionts associated with obligate blood feeders; Blue, intracellular
542 symbionts of other arthropods; Black, intracellular pathogens of mammals. Filled arrows and
543 white arrows indicate intact genes and pseudogenes, respectively.

544 **Table 1. Biosynthetic pathways for B vitamins in 11 KEGG curated* genomes of symbionts associated with OBF.**

B vitamin provisioning symbiont				Obligate blood feeder host	KEGG code	Vitamin B ₁ Thiamine	Vitamin B ₂ Riboflavin	Vitamin B ₃ Nicotinic acid	Vitamin B ₅ Pantothenic acid	Vitamin B ₆ Pyridoxine	Vitamin B ₇ Biotin	Vitamin B ₉ Folate	Vitamin B ₁₂ Cobalamin
Phyla	Order	Family	Strain										
γ-proteobacteria	Legionellales	Coxiellaceae	<i>Coxiella</i> str. CRT	Tick (<i>Rhipicephalus turanicus</i>)	cey	□	■	■	■	■	■	■	□
			<i>Coxiella</i> str. CeAS-UFV	Tick (<i>Amblyomma sculptum</i>)	cend	■	■	■	■	■	■	■	□
			<i>Coxiella</i> str. CLEAA	Lone star tick (<i>Amblyomma americanum</i>)	cea	■	■	■	■	■	■	■	□
γ-proteobacteria	Thiotrichales	Francisellaceae	<i>Francisella persica</i>	Soft tick (<i>Argas arboreus</i>)	fper	□	■	■	■	■	■	□	
γ-proteobacteria	Enterobacterales	Erwiniaceae	<i>Wigglesworthia glossinidia</i>	Tsetse fly (<i>Glossina brevipalpis</i>)	wbr	■	■	■	■	■	■	■	□
			<i>Wigglesworthia glossinidia</i>	Tsetse fly (<i>Glossina morsitans</i>)	wgl	■	■	■	■	■	■	■	□
γ-proteobacteria	Enterobacterales	Morganellaceae	<i>Arsenophonus lipopteni</i>	Deer keds (<i>Lipoptena fortisetosa</i>)	asy	□	■	□	□	■	■	□	□**
γ-proteobacteria	Enterobacterales	Enterobacteriaceae	<i>Cand. Riesia</i> sp. GBBU	Gorilla louse (<i>Pthirus gorillae</i>)	rig	□	□	□	□	□	■	□	□
			<i>Cand. Riesia pediculicola</i> str. USDA	Human body louse (<i>Pediculus humanus humanus</i>)	rip	□	■	■	■	■	■	■	■
α-proteobacteria	Rickettsiales	Midichloriaceae	<i>Cand. Midichloria mitochondrii</i>	Castor bean tick (<i>Ixodes ricinus</i>)	mmn	□	□	□	□	□	■	■	□
α-proteobacteria	Rickettsiales	Anaplasmataceae	<i>Wolbachia</i> str. wCle	Bed bug (<i>Cimex lecturalis</i>)	wcl	■	■	□	□	■	■	■	□

545

546 *KEGG pathways used for B₁, B₂, B₃, B₅, B₆, B₇, B₉ and B₁₂ are 00730, 00740, 00760, 00770, 00750, 00780, 00790, 00860, respectively. Black squares,

547 putatively functional pathways; Grey squares, incomplete pathways with pseudogenes or missing genes; white squares, pathways absent.

548 ** Only flavin reductase (*fre*) present. An enzyme that converts Aquacob(III)alamin into vitamin B₁₂.

549