# Caught in the act: Rapid, symbiont-driven evolution

## Endosymbiont infection is a mechanism generating rapid evolution in some arthropods – but how widespread is the phenomenon?

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**Facultative bacterial endosymbionts can transfer horizontally among lineages of their arthropod hosts, providing the recipient with a suite of traits that can lead to rapid evolutionary response, as has been recently demonstrated. But how common is symbiont-driven evolution? Evidence suggests that successful symbiont transfers are most likely within a species or among closely related species, although more distant transfers have occurred over evolutionary history. Symbiont-driven evolution need not be a function of a recent horizontal transfer, however. Many endosymbionts infect only a small proportion of a host population, but could quickly increase in frequency under favorable selection regimes. Some host species appear to accumulate a diversity of facultative endosymbionts, and it is among these species that symbiont-driven evolution should be most prevalent. It remains to be determined how frequently symbionts enable rapid evolutionary response by their hosts, but substantial ecological effects are a likely consequence whenever it does occur.**

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## Introduction

There was once a time when ecologists could pursue their discipline without undue concern over evolutionary processes, secure in the paradigm that ecological and evolutionary dynamics took place on different timescales [1], and that evolutionary change occurs too slowly to be relevant to the here-and-now of ecological communities. No more. In recent decades, we have seen numerous examples of contemporary evolution [2], and the rise of ''community genetics'' and ''eco-evolutionary dynamics'', which emphasize the dynamic interplay of ecological and evolutionary processes [2, 3]. The potential for ecologically relevant evolution becomes even more pronounced when organisms are capable of wholesale acquisition of novel traits in a single generation. This phenomenon is well-described among prokaryotes, where horizontal gene transfer is responsible for widespread dissemination of functional traits (e.g. antibiotic resistance in bacteria [4]). It is now evident that multicellular eukaryotes, too, can make a sudden leap to a new peak in the adaptive landscape: horizontal transfer of a bacterial endosymbiont (Box 1) can confer a ready-made suite of characteristics upon the host, which in turn could have substantive consequences for the ecological community [5].

Himler et al. [6] recently described the spread of a *Rickettsia* bacterium through populations of the sweet potato whitefly (*Bemisia tabaci*) in the southwestern United States. Infection by the bacterium was shown to confer substantial fitness benefits to the host: in laboratory assays, infected whiteflies had 15–30% higher survival to adulthood, developed to adulthood 1–2 days faster, produced twice as many offspring, and produced a greater proportion of female offspring than uninfected counterparts. The mechanism by which *Rickettsia* caused these effects was unclear, but the result was a strong selective advantage for infected hosts. Near-perfect vertical transmission of the bacteria, plus this selective advantage, caused *Rickettsia* infection to ''sweep'' through populations of the sweet potato whitefly, going from a

## Box 1

**Life on the inside**

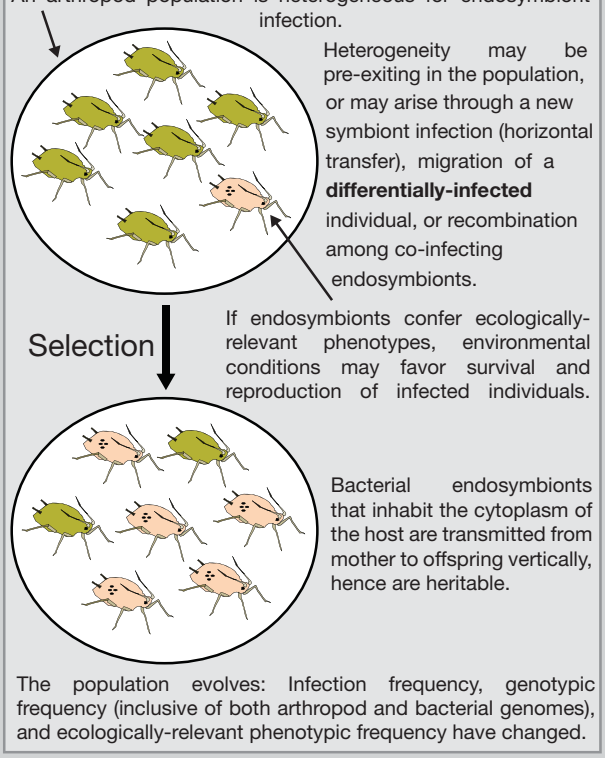
Bacteria that reside inside hosts (**endosymbionts**) may be either **intracellular** or **extracellular** [49]. Intracellular symbionts have gained access to a benign environment in the cytoplasm, but no longer have easy access to the outside world, and share the fate of their host organism. These bacteria are therefore under selection to (i) maximize benefit and minimize cost to their host, and (ii) ensure transmission to future host generations.

Some bacteria provide essential (usually nutritive) functions for their host, and are **obligate** from the host's perspective [11], whereas others are not strictly necessary, and are considered **facultative**. Obligate and facultative intracellular bacteria differ qualitatively in a number of respects. In general, obligate intracellular bacteria have extremely reduced genomes [12], are housed in specialized tissues (**bacteriomes**) and rely exclusively on **vertical** transmission from mother to offspring, typically within the egg itself (**transovarial** transmission). Facultative bacteria have somewhat more independence from the host, retaining a larger genome characterized by a large proportion of mobile DNA [50], and inhabit a wider range of host tissues [51]. Consequently, facultative endosymbionts have greater opportunity and ability to undertake **horizontal** transmission among unrelated individuals. Both intraspecific and interspecific horizontal transmission have been documented [28, 29, 52].

Facultative bacterial endosymbionts are often not fixed in the host population, persisting at a frequency of $<1$. In part, this is because they tend to confer **conditionally beneficial** phenotypes that are selectively favored in some environments, but not others. Additionally, many facultative endosymbionts engage in **reproductive manipulation** of their host. For maternally transmitted bacteria, males are dead ends, and it is in the endosymbiont's interest to promote production of females by the host. Reproductive manipulations that accomplish this can spread infection through a host population, even if infection exerts a cost on the host. However, because the vertical transmission efficiency of facultative symbionts is often less than perfect, the outcome will be an equilibrium infection frequency of $<1$ [53].

## Box 2

**Symbiont-driven evolution**




frequency of near zero to near fixation in less than six years (Box 2). Symbiont sweeps have been documented in two other systems [7, 8]. One of these was also associated with a highly advantageous phenotype for infected individuals (defense against nematodes [8]), similar to *Rickettsia* in whiteflies, whereas the other was caused by the unique evolutionary dynamics of cytoplasmic incompatibility (Table 1) which led to symbiont spread despite fitness costs to the host [7, 9].

These studies have demonstrated that bacterial endosymbionts *can* drive rapid evolution of insects, with inevitable consequences for the ecological community [10]. What remains to be seen is whether these examples are indicative of a pervasive pattern: Is symbiont-driven evolution a rare event, or a common occurrence? If common, hidden microbial players may be having a larger effect on contemporary ecological interactions than we ever imagined.

## Facultative symbionts are prevalent among arthropods, and often confer conditionally beneficial traits

Endosymbiotic interactions between arthropods and microorganisms can range from pathogenic to mutualistic, and may be either obligate or facultative (from both host and microbe perspectives) [11]. For the purposes of this essay, however, the foci are symbioses that are facultative from the host's perspective but obligate from the microbe's perspective (referred to hereafter as facultative endosymbioses), because these are the symbioses that have the most potential for rapid evolutionary impact. Endosymbionts that are obligate from the host's perspective have often spent millions of years coevolving with their hosts [12], usually have strict vertical transmission with little opportunity for horizontal transfer, and would be unlikely to survive outside their coevolved host environment (Box 1). In contrast, microbes that only facultatively utilize host arthropods, or that have widespread horizontal (virulent) transmission among hosts, do not share a common interest with a particular host and thus are under little selective pressure to confer large phenotypic effects [13],

**Table 1. Host phenotypes associated with intracellular facultative bacterial endosymbionts**

| Host Phenotype | Description (taxa affected) |
|---|---|
| **Reproductive manipulations** | |
| Cytoplasmic incompatibility | Matings between infected males and uninfected females have reduced or zero fertility (many arthropods). |
| Feminization | Genetic males are modified into phenotypic females (isopods, mites). |
| Parthenogenesis | Female lineages produce female offspring without mating (parasitic wasps). |
| Male-killing | Male offspring are killed, providing more resources for female siblings (ladybird beetles, moths, parasitic wasps). |
| **Defense** | |
| Against viruses | Endosymbiont-infected hosts show improved survival (*Drosophila*). |
| Against fungi | Endosymbiont-infected hosts (aphids) show improved survival. |
| Against nematodes | Endosymbiont-infected hosts (*Drosophila*) retain fertility despite infection by castrating nematodes. |
| Against arthropods | Parasitoid wasps fail to develop in endosymbiont-infected hosts (aphids). |
| **Other** | |
| Thermal tolerance | Survival of endosymbiont-infected hosts (aphids) improved at high temperatures. |
| Host plant interactions | Endosymbionts affect the plant range of hosts (aphids) or allow host to modify plant physiology (moth). |
| Protective coloration | Endosymbiont changes protective coloration of host (aphid) as host ages. |
| Competency as a vector | Infected individuals show increased (whiteflies) or decreased (flies) propensity to vector viruses. |

nor would any such effects be considered heritable. Between these extremes lie the facultative endosymbionts, which are primarily transmitted vertically (thus are heritable, and under pressure to provide a benefit to their host), but retain some capacity for horizontal transfer.

Despite the restrictive definition, vertically transmitted facultative endosymbionts are widespread among arthropods, including bacterial, viral, microsporidian, and fungal microbes [14–16]. Focusing on bacteria, facultative endosymbionts are present in taxa from most arthropod lineages (Table 2). Estimates of facultative endosymbiont infection frequency are constantly being upgraded as new symbionts are characterized, and as improved molecular detection techniques have increased detection of low-titer, cryptic and rare

**Table 2. Distribution of intracellular facultative bacterial endosymbionts among arthropods**

| Taxa | Recorded symbionts[a] |
|---|---|
| **Arachnida** | |
| Araneae (Spiders) | *Ars*, *Card*, *Rick*, *Spir*, *Wol* [46, 54][b] |
| Acari (Mites and Ticks) | *Ars*, *Card*, *Rick*, *Rickettsiel*, *Spir*, *Wol* [55–60] |
| Opiliones (Harvestmen) | *Card* [57] |
| Scorpionida (Scorpions) | *Wol* [61] |
| **Crustacea** | |
| Isopoda (pillbugs) | *Wol* [46] |
| Amphipoda (amphipods) | *Wol* [62] |
| **Hexapoda** | |
| Collembola (springtails) | *Rick*, *Wol* [63, 64] |
| Protura | *Card* [65] |
| Insecta | |
| Odonata (dragonflies) | *Wol* [66] |
| Orthoptera (grasshoppers) | *Wol* [54, 67] |
| Dictyoptera (roaches, termites, mantids) | *Ars*, *Wol* [55, 63] |
| Neuroptera (lacewings, antlions) | *Ars*, *Rick*, *Spir*, *Wol* [55, 56, 58, 68] |
| Coleoptera (beetles) | *Ham*, *Rick*, *Sod*, *Spir*, *Wol* [46, 54, 56, 58, 69] |
| Strepsiptera (twisted wing parasites) | *Wol* [70] |
| Diptera (flies) | *Ars*, *Card*, *Rick*, *Sod*, *Spir*, *Wol* [46, 54–58] |
| Siphonaptera (fleas) | *Rick*, *Wol* [58, 71] |
| Lepidoptera (butterflies and moths) | *Rick*, *Spir*, *Wol* [54, 56, 58] |
| Hymenoptera (bees, wasps, ants) | *Ars*, *Card*, *Rick*, *Serr*, *Spir*, *Wol* [54–58,72] |
| Psocodea (lice) | *Rick*, *Wol* [58, 71] |
| Thysanoptera (thrips) | *Wol* [68] |
| Hemiptera (true bugs, sucking plant insects) | *Ars*, *Card*, *Ham*, *Reg*, *Rick*, *Rickettsiel, Serr*, *Sod*, *Spir*, *Wol* [41, 43, 54–58,73] |

[a] Not an exhaustive list. Symbionts that have been described from only a single host, for which a generic designation has not been made, or that lack confirmation of intracellular status (in at least some host species) are not included.

[b] Abbreviations indicate endosymbiont genera. *Ars*: *Arsenophonus* (gamma-proteobacteria), *Card*: *Cardinium* (Bacteroidetes), *Ham*: *Hamiltonella* (gamma-proteobacteria), *Reg*: *Regiella* (gamma-proteobacteria), *Rick*: *Rickettsia* (alpha-proteobacteria), *Rickettsiel*: *Rickettsiella* (gamma-proteobacteria), *Serr*: *Serratia* (gamma-proteobacteria), *Sod*: *Sodalis* (gamma-proteobacteria), *Spir*: *Spiroplasma* (Mollicutes), *Wol*: *Wolbachia* (alpha-proteobacteria).

infections [17, 18]. *Wolbachia*, the most prevalent of these bacteria, is currently estimated to infect >65% of arthropod species, although often at low infection frequency within a species [19]. Historically, *Wolbachia* and some other facultative endosymbionts have been categorized as reproductive parasites [9], because the first and most common phenotypes described for these bacteria were reproductive manipulations that propagate bacterial infection without providing a benefit to infected hosts (Table 1, 3). The phenotypic shifts brought about by reproductive manipulators (e.g. male-killing) can have substantial ecological consequences in their own right [20], but it is worth noting that reproductive manipulators, too, are under selective pressure to minimize costs to their hosts, and evolutionary shifts from parasite to mutualist have been documented [21]. Furthermore, *Wolbachia* has recently been shown to provide defense against viruses in some host lineages [22, 23]. Some authors have now begun to question whether reproductive manipulation is necessarily the primary modality of these bacteria [18].

Of the more mutualistic phenotypes conferred by facultative bacterial symbionts (Table 1), most are conditionally beneficial, as might be expected by analogy from the prevalence of conditionally beneficial traits encoded by mobile genetic elements [24]. For example, bacterial defense against parasitoids is only beneficial in environments where parasitoids are present; in the absence of parasitoids, infected hosts have been shown to be at a disadvantage relative to uninfected counterparts [25]. Balancing selection therefore is likely to maintain such symbionts at a frequency of less than 1, in a mosaic across the landscape [26]. Having a mixture of differentially infected individuals increases the range of phenotypes present in the host population, and likely contributes to the host's overall responsiveness to selection. If one considers the combined genetic variation (inclusive phenotype) that is generated by a host and its symbionts, then the reservoir of available genetic variation available for natural selection to work upon is amplified over what is available based on host genetic material alone. Indeed, some authors have likened facultative symbionts to "accessory genomes" that confer functional "macromutations" on their hosts [27].

## Rapid evolutionary response may be a function of facultative endosymbiont diversity

Ultimately, a host's ability to benefit from symbiont-based variation depends on the probability of acquiring functional endosymbionts through horizontal transfer. Empirical studies have illustrated that symbiotic transfer can occur between intimately associated species [28, 29], and that certain habitats and lifestyles tend to facilitate horizontal transmission of facultative endosymbionts [30, 31]. Phylogenetic analyses also provide ample evidence of historic horizontal transfers, even among distantly related taxa [32]. However, these studies also give evidence of co-cladogenesis in some lineages and/or show taxonomic affinity between some groups of hosts and

**Table 3. Initial documentation of host phenotypes induced by intracellular facultative bacterial endosymbionts of arthropods**

| | Symbiont genera | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Ars*[a] | *Card* | *Ham* | *Reg* | *Rick* | *Rickettsiel* | *Serr* | *Sod* | *Spir* | *Wol* |
| **Reproductive manipulation** | | | | | | | | | | |
| Cytoplasmic incompatibility | | 2003 [74] | | | | | | | | 1973 [75] |
| Feminization | | 2001 [76] | | | | | | | | 1973 [77] |
| Parthenogenesis | | 2004 [78] | | | 2006 [79] | | | | | 1990 [80] |
| Male killing | 1986 [81] | | 2010 [69] | | 1994 [82] | | | | 1999 [83] | 1999 [84] |
| Female biased sex ratio[b] | | | | | 2011 [6] | | | | | 2003 [85] |
| **Defense** | | | | | | | | | | |
| Against viruses | | | | | | | | | | 2008 [22, 23] |
| Against fungi | | | | 2005 [86] | | | | | | |
| Against nematodes | | | | | | | | | 2010 [8] | |
| Against arthropods | | | 2003 [87] | 2010 [88] | | | 2003 [87] | | 2010 [89] | |
| **Other** | | | | | | | | | | |
| Thermal tolerance | | | | | 2011 [39] | | 2006 [90] | | | |
| Host plant interactions | | | | 2004 [91][c] | 2010 [92][d] | | | | | 2010 [93] |
| Protective coloration | | | | | | 2010 [41] | | | | |
| Competency as a vector | | | 2010 [94][d] | | | | | | | 2010 [95] |
| Increased reproduction and/or longevity[b] | | 2004 [96] | | | 2011 [6] | | | 2001 [97] | | 2002 [98] |

[a] Refer to Table 2 for abbreviations.
[b] Mechanism unknown.
[c] But see ref. [99, 100].
[d] Based on comparisons among differentially infected populations, without experimental manipulation of infection.

endosymbionts [33, 34], suggesting a certain amount of host specialization on the part of the bacteria. Host background has been shown to affect phenotypic expression by endosymbiotic bacteria: for example, a *Wolbachia* strain that kills males in its original host caused cytoplasmic incompatibility when transferred to a new host [35]. Likewise, experimentally transinfected bacteria often fail to become stably established across multiple generations in the new host species, particularly if the donor and recipient host species are only distantly related [34], probably because of poor adaptation of the bacteria to the new host environment. Among closely related host taxa, the probability of generating stable infections is improved, with some recipient host lines remaining stably infected for many generations following transinfection [34]. This pattern suggests that natural horizontal transfer may occur regularly within or among closely related species.

It is important to recognize, however, that while horizontal transfer of symbionts provides the fodder for natural selection and the potential for rapid evolutionary response, such evolutionary responses are not necessarily coincident with the initial infection event. For example, the *Spiroplasma* endosymbiont that confers resistance against *Howardula* nematodes was present, but not prevalent, in *Drosophila neotestacea* collected more than 20 years ago [8]. Perlman and Jaenike hypothesized that the recent sweep of *Spiroplasma* through *D. neotestacea* populations was prompted by the recent colonization of N. America by a new species of nematode, *H. aoronymphium* [36]. Symbionts without immediate benefit to the host may be able to persist at low infection frequency in host populations for long periods of time as essentially neutral passengers if their vertical transmission rate is high and they do not strongly affect host fitness [37]. Thus, perhaps it is the available diversity of symbionts within a host species, rather than recent acquisition of a new symbiont, that would best predict the propensity for rapid evolutionary adaptation.

If symbiont diversity increases genetic variation, facilitates adaptation, and can even lead to rapid evolution of a host, then it is not surprising that symbiont-driven evolution has been documented in the sweet potato whitefly [6]. This widespread pest is composed of genetically and ecologically distinct biotypes that are differentially infected with multiple strains of at least six different facultative symbionts [38]. It would be premature to attribute the ecological distinctiveness of the various biotypes to their symbionts, but the recent study by Himler et al. [6], as well as correlative studies among differentially infected strains [39], lends credence to this possibility. Similarly, the pea aphid, *Acyrthosiphon pisum*, is a widespread pest that hosts multiple facultative endosymbionts that confer ecologically relevant phenotypes [40, 41], again suggesting a role for symbionts in the adaptation and ecological divergence of the species. However, it is also possible that the symbiont diversity exhibited by these species is merely a consequence of their widespread distribution, rather than a cause.

Furthermore, it has yet to be established that the symbiont diversity exhibited by these species is even particularly noteworthy. There are several reasons to believe that we substantially underestimate symbiont diversity in most host taxa. First, the probability of detecting infections within a species increases with sampling effort [19], and relatively few host species have been sufficiently scrutinized to detect low frequency endosymbiont infections. For example, most of the symbionts described in the pea aphid are also present in multiple other aphid species [42, 43]. However, since only a single specimen has been screened in most aphid species, the frequency and diversity of endosymbiont infection cannot be evaluated within these species. Second, as more rigorous criteria for differentiating endosymbionts strains are developed and deployed [44], it is becoming evident that cryptic infections of a host species by multiple strains of the same symbiont are not uncommon [18, 38], and that these strains can differ in the phenotypic effects conferred on the host [45]. Third, co-infections of the same individual by multiple endosymbionts are being discovered more frequently [46, 47]. Such multiple infections provide opportunities for recombination among endosymbionts via phages and other mobile elements [48], further increasing symbiont diversity within the host population.

## Conclusions

Until a great deal more data are available on the distribution and diversity of facultative endosymbionts within host species, it is impossible to evaluate whether high symbiont diversity is a property of a few exceptional species, or much more widespread. To date, studies on facultative endosymbionts in arthropods have largely focused on endosymbiont phylogeny, the types of phenotypes conferred (Table 1), and the mechanisms by which they act, with only rare snapshots being taken of symbiont diversity in the field [17, 18, 46, 47]. A shift in emphasis toward understanding the population and evolutionary dynamics of facultative symbionts in natural populations will allow us to begin to understand the ecological consequences of endosymbiont infection, the short- and long-term evolutionary trajectories they mediate, and the adaptation and ecological differentiation of host taxa. Understanding these factors may give us insight into species' responses to environmental change, extinction risk, invasive potential, and pest status. Once we start looking, we may find that rapid symbiont-driven evolutionary responses, such as that described by Himler et al., are commonplace.

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