Reproductive Parasitism and Positive Fitness Effects of Heritable Microbes

ID: A28327

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Abstract

The classification of host-symbiont relationships is usually defined along the parasitism-mutualism spectrum. It has long been proposed that transmission route is a key factor driving this, with vertical transmission leading to mutualism and horizontal transmission leading to parasitism. However, uniparental vertical transmission can lead to the evolution of reproductive parasitism, whereby host reproduction is skewed to increase the proportion of females within a population or else to reduce the comparative fitness of uninfected females (to the detriment of overall host fitness). Once discussed separately from beneficial effects and mutualism, we now recognise reproductive parasitism is not exclusive of other symbiont phenotypes. We outline the evolution and relationship of reproductive parasitism with respect to positive fitness effects for hosts, and how these interactions may be dynamic across the parasitism-mutualism continuum.

Key Terms

Reproductive parasite | Heritable microbe | Vertical transmission | Sex ratio distorter | Conditional sterility | Fitness benefit | Cost-Benefit | Obligate mutualism | Facultative mutualism | Uniparental inheritance

Key Concepts

I. *Exclusive maternal transmission of microbes can create strong selection for reproductive parasitism.*
II. *Heritable microbes are also selected to confer a range of positive effects on host function and physiology*
III. *Where heritable microbes act both as reproductive parasites, and as a positive influence on host function, they are referred to as Jekyll and Hyde symbionts.*
IV. *The presence of positive effects on host function can facilitate the invasion and maintenance of reproductive parasites in host populations.*
V. *Reproductive parasitism may likewise provide a context in which symbionts may evolve host-beneficial phenotypes.*
VI. *Symbionts that combine reproductive parasitism with positive effects on host function constitute a useful mechanism for modification of insect host biology in natural populations, coupling a strong gene drive system to a beneficial trait.*
VII. *The presence of multiple phenotypes may aid the spread of heritable microbes through host communities, by enabling host shift events.*
VIII. *Lateral transfer of genetic information between microbes can provide the mutational mechanism through which Jekyll and Hyde symbionts arise.*

Introduction

Heritable microbial symbionts - bacteria, viruses and fungi that are transmitted from parent to offspring - are common in nature and constitute an important part of host biology. Widely present in invertebrate animals, plants and fungi, vertically transmitted microbes are predominantly maternally inherited, passing from mother to offspring. This matrilineal pattern is associated with asymmetry in both gamete size (the small size of sperm heads commonly exclude microbial symbionts), and in the contact between parent and offspring (common for females, less common for male hosts). Indeed, whilst there are many accounts of maternally inherited bacteria, paternal inheritance is rarely documented (see De Vooght *et al*., 2015 for exception).

In some cases, these microbes manipulate host reproduction to facilitate their own transmission (through female hosts) and drive through populations. These cytoplasmically transmitted elements have become known as reproductive parasites (RP) and include members of the bacterial genera *Wolbachia*, *Rickettsia*, *Arsenophonus* (Proteobacteria), *Cardinium*, *Flavobacteria* (Bacteroidetes), *Spiroplasma* (Firmicutes), the eukaryotic Microspora, and certain viruses. The individual impact of these symbionts feeds through to important population level consequences, with reproductive parasites driving rapid natural selection and contributing to speciation.

Carrying this class of heritable microbe can be detrimental to infected host individuals. However, there is growing evidence that the evolution of positive effects on host function and physiology are important for the spread and maintenance of heritable microbes in host populations. Importantly, heritable microbes classically associated with reproductive parasitism are increasingly found in the absence of reproductive manipulation phenotypes, indicating that these symbionts are highly likely to have direct beneficial impacts on their host. For instance, there was a period of time when 'no effect' *Wolbachia* were widely discussed. This 'no effect' related to the absence of reproductive parasitism – but this absence as a corollary indicates the presence of direct beneficial effects of symbiont infection – else the infection would not drive into and be maintained in the population. Indeed, some symbionts combining beneficial and reproductive parasitic phenotypes have now been described – and termed 'Jekyll and Hyde' infections. (See also: DOI: 10.1002/9780470015902.a0000390.pub3, DOI: 10.1038/npg.els.0001758)

Routes to Invasion

For any heritable symbiont to spread within a host population, the 'drive' to spread must outweigh any metabolic or pathological costs of infection, as well as any segregational loss (failure to inherit). Vertical transmission creates an association between symbiont fitness and host fitness - what is good for the host is good for the symbiont. This association may lead to selection on the symbiont to promote host survival and reproduction i.e. beneficial symbiont phenotypes. For example, *Hamiltonella defensa* drives itself into populations by protecting its aphid hosts from parasitoid wasp attack. Protective symbionts such as these increase the chances that their host will survive to reproduce in comparison to uninfected hosts, driving the spread of infection.

Nutritional contributions are another common beneficial phenotype, as larger and better nourished individuals will sire more offspring. Such anabolic contributions are integral to the evolution of many host-symbiont interactions. Indeed, the majority of phloem and blood feeding insects depend on symbionts, providing the host with essential amino acids or vitamins that are not otherwise found in their nutrient poor diets. In these situations, it is logical that selection acts to maintain the mutualism between host and symbiont. (See also: DOI: 10.1002/9780470015902.a0028127)

However, vertical transmission is commonly restricted to one host sex, with maternal inheritance most common. When a symbiont is only transmitted vertically by one sex the evolutionary forces on the symbiont become more complex than the simple model of host fitness being aligned with that of the symbiont. For maternal inheritance, the fitness of the microbe relates solely to the survival and production of female hosts. Reproductive manipulation phenotypes can evolve in these situations. (See also: DOI: 10.1038/npg.els.0001745, DOI: 10.1002/9780470015902.a0005444.pub3).

Reproductive Manipulation by Heritable Microbes

Maternally inherited microbes can only be transmitted via the female line, therefore male hosts constitute evolutionary 'dead ends' for these symbionts (Cosmides and Tooby, 1981). Where the symbiont is capable of infectious transmission, selection may favour sacrifice of male hosts for infectious transmission whilst maintaining female hosts for vertical transmission. Where infectious transmission is not possible, uniparental inheritance has led to the evolution of mechanisms to manipulate host reproduction in favour of the production or survival of female hosts. Two major strategies are observed: distorting sex ratios of infected hosts towards female hosts and inducing conditional sterility (see Figure 1).

One of the first sex ratio distorting phenotypes recorded was male-killing, where particular matrilines produce both male (sons) and female offspring (daughters), but sons die before maturity. This single phenotype has two drivers. First, where infectious transmission through the environment is possible (Microspora, certain viruses), male larvae are killed to enable dispersal of the microbe to infect new hosts whilst females are retained for vertical transmission (Figure 1A). Second, where infectious

transmission is constrained, male death occurs during embryogenesis (Figure 1B). Killing male embryos releases resources to sibling female hosts that carry the same symbiont, and thus increases the survival of host individuals that can transmit the symbiont (females) above those that cannot (the males) (Werren 1987; Hurst 1991; Hurst and Majerus, 1993).

Male-killing is a weak form of drive, in that it represents a reallocation of resources to infected females rather than an increase in the absolute number of females formed. Stronger drive is presented in cases of induction of female biased primary sex ratios, where female individuals are produced at the expense of male. Female biased primary sex ratios are known to be promoted through inducing either host parthenogenesis (Figure 1C) or feminizing males that are produced (Figure 1D). In the former, sons are not produced. In the latter, sons are produced but are converted to a female phenotype during development.

Whilst sex ratio distortion is a relatively simple phenotype to understand in terms of logic, cytoplasmic incompatibility (CI), the most common of the manipulation phenotypes, is a more subtle phenotype. CI represents a type of conditional sterility, where only females that are either uninfected, or infected with a different strain, are impacted (Figure 1E). The phenotype exists in two forms, both of which lead to mating incompatibilities. In unidirectional CI, crosses between uninfected females and infected males leads to mortality of up to 100% of embryos (Engelstädter and Hurst, 2009). Bidirectional CI generates incompatibilities between egg and sperm when each partner carries a different strain of the reproductive parasite. In both cases the incompatibility can be rescued if the egg carries the same symbiont strain as the sperm (Werren, 1997). For the mechanistic basis of reproductive manipulation phenotypes, see Table 1. (See also DOI: 10.1038/npg.els.0001714)

The genes which induce reproductive manipulation are by definition selfish genetic elements, since these manipulations are deleterious to their host. Feminisation and parthenogenesis-induction increase the number of females in the population which can transmit infection, but both have an overall negative impact on host fitness. Feminised males are known to be less reproductively fit than genetic females and parthenogenetic populations are more susceptible to extinction due to reduced population genetic diversity. Whilst male killing can benefit the infected mother in terms of increased fitness of daughters, this is counteracted by the greater loss of sons. CI involves loss of

male fertility during the spread phase (although when at high frequency, it is beneficial for a female to retain the symbiont to protect itself against CI). In all cases it can be assumed that at the very least these bacteria induce a metabolic burden on their host and are thus often considered parasitic with respect to the mutualism-parasitism continuum (Reviewed in Zug and Hammerstein, 2015).

Reproductive Parasites as Gene Drive Systems

Like other selfish elements, the manipulation phenotypes of reproductive parasites can drive these elements through populations at much faster rates than standard selection on nuclear genes. In under ten years a CI inducing *Wolbachia* spread over 700km in *Drosophila simulans* populations, despite lowering the fecundity of infected females (Turelli and Hoffmann 1991; Weeks *et al.*, 2007). Rapid spread of a *Rickettsia* sex ratio distorter was recently observed in US populations of *Bemisia tabaci*, with the symbiont sweeping to near fixation (97 % infection frequency) in under 6 years (Himler *et al.*, 2011). These agents spread under very strong selection, and contemporary spread is observed relatively commonly in insect populations.

Evolution of Mutualism and Reproductive Parasitism

Whilst many reproductive manipulators were once defined solely by the RP phenotype that they induce, it is now becoming clear that the presence of a reproductive parasitic phenotype is not mutually exclusive to the symbiont having a positive fitness effect on the host (Table 2). For instance, the male-killing *Spiroplasma* of *D. melanogaster* additionally provides defence to its host against attack by parasitic wasps (Xie *et al.*, 2014; Paredes *et al.*, 2016). The sex ratio distorting *Rickettsia* of *B. tabaci* has a range of positive effects on host survival, development and fecundity (Himler *et al.*, 2011). Strains of *Wolbachia* that produce CI additionally produce protection against ssRNA virus attack (Hedges *et al.*, 2008). The direct benefits conferred to hosts can take a myriad of forms, as highlighted in Table 2, and include nutrient provisioning, environmental tolerance, reproductive benefits and protection against natural enemies. Indeed, selection promotes

any aspect of the symbiont which contributes to the production and survival of daughters – both via reproductive parasitism and through impacts on the biology of female hosts.

Beneficial Effects and Invasion of Reproductive Parasites

We have discussed how reproductive manipulations are substantial drivers of infection into populations. However, it is clear that direct fitness benefits to infected hosts are also a significant contributor to the invasion success of RPs. Previously this contribution has been overlooked, but it is increasingly apparent that mechanisms of reproductive parasitism alone cannot always account for symbiont invasion (Zug and Hammerstein, 2018). Beneficial effects can aid the spread of all reproductive parasites within a population as fitter infected females would produce more daughters to spread infection. For male-killing, this impact may allow invasion of strains where the drive from male-killing is weak, for instance where male death has only a small impact on female sibling survival. With a beneficial effect of infection, symbionts with even low levels of drive through male-killing could persist (Zug and Hammerstein, 2015). These effects may explain enigmatic cases of male-killing in hosts such as *Danaus chrysippus*, where the female lays eggs singly, and thus there is little interaction between siblings (Jiggins *et al.*, 2000).

However, beneficial effects are likely of particular importance to the invasion of CI inducing strains, since here the reduction of fitness to uninfected individuals is dependent upon the number of infected males in the population (i.e. the effects of CI are positively frequency dependant). At the point of introduction of the symbiont, the drive from CI alone is very weak – the fraction of infected males is the reciprocal of the population size of males – such that models where infection has no benefit predict CI strains must reach a threshold frequency before they can invade. Beneficial effects that sit alongside CI can allow the symbiont to invade even when rare (Fenton *et al.*, 2011). In many cases this invasion from low frequency would not be possible when CI is a stand-alone phenotype (Zug and Hammerstein, 2018). Once established above the threshold frequency, the CI phenotype takes the symbiont strain to very high frequency.

RP Drive and Beneficial Effects have Important Applications

As noted previously, the presence of a RP effect can represent a substantial drive enabling the invasion of a host species by a symbiont. Where this symbiont has desired characteristics, these are then acquired additionally. This synergy has been harnessed to alter vector competence in *Aedes aegypti* mosquitoes. *Wolbachia w*Mel has been transinfected into the mosquito, where it both causes CI and reduces host competence for transmission of RNA viruses such as those causing dengue (Walker *et al.*, 2011). The CI trait enables rapid invasion and maintenance in mosquito populations following mass release, and the symbiont at equilibrium impairs mosquito competence. This strategy has been used to provide effective public health benefits in Northern Australia and interest is growing in its application for the control of other emerging arboviral diseases (Moreira *et al.*, 2009; Hoffmann *et al.*, 2011; O'Neill *et al.*, 2018)

Beneficial Effects and the Maintenance of Symbionts

Once a reproductive manipulator is at an equilibrium in a host population, selection acts on both host and symbiont with respect to that symbiosis (see Figure 2). For the symbiont, selection will act to reduce costs of infection, and indeed provide benefit to the particular host species into which it has spread. Reproductive parasitism may thus enable the evolution of beneficial symbioses, through driving an infection to equilibrium which is then selected to benefit the host species in which it is found.

Selection also acts upon the host. First, there is selection to prevent the reproductive parasitic action of the symbiont. If a strain exhibits RP then this commonly selects on the host for the evolution of resistance to the symbiont (eg. Hornett *et al.*, 2006). In this situation, symbiont maintenance is made more likely by the presence of alternate beneficial phenotypes. Second, the host may be selected to tolerate the symbiont – that is to say to modify its biology to either reduce the costs of infection, or to otherwise promote fitness given the symbiont is present.

Tolerance to a reproductive manipulator does not create beneficial effects but alleviates the negative effects induced by symbiont infection. Once tolerance has evolved,

dependence upon the symbiont may follow. For *Asobara tabida*, a species of parasitoid wasp, one of the three *Wolbachia* strains is obligately required for egg cell development (Kremer *et al.*, 2009), but closely related host species do not share this dependence. Removal of *Wolbachia* initiates atypical apoptosis, suggesting an evolved dependence on the parasite that does not truly benefit the host. Removal of the parasite will negatively impact host fitness, such that this evolved obligate dependency may be mistaken for a mutualistic interaction (Werren, 2011). Indeed in many cases interactions that appear to benefit hosts now appear likely to represent the evolution of tolerance by the host in an effort to mitigate costs.

All these processes- selection on the symbiont for benefit, selection on the host to ablate reproductive parasitism, and selection on the host to tolerate infection, means these symbioses move rapidly over the benefit-parasitism continuum (see Figure 2). (See also: DOI: 10.1002/9780470015902.a0028127).

'Jekyll and Hyde' Symbionts in a Community Context

Whilst symbionts displaying reproductive parasitism are common, it is rare to observe pairs of closely related species where both carry the same symbiont by virtue of descent from a shared ancestor. Rather, the widespread presence of heritable microbes across host species is a result of host shift events, where a symbiont is introduced from one host species to another, subsequently invading the novel host species. Evidence from the presence of very closely related symbionts in evolutionarily more distant hosts implies these host shifts occur quite commonly (Turelli *et al.*, 2018). The converse of this rapid rate at which new host-symbiont combinations establish is that they must be relatively short lived within a particular species – they do not infect all species, and rarely infect sibling species pairs by descent.

The capacity for host shift events is thus a key determinant of heritable microbe incidence. The presence of multiple phenotypes – beneficial and RP effects – may be important for persistence by enabling spread through a wider variety of host species. The benefits to RP phenotypes, or the capacity to achieve them, varies with the host species. In some species, male-killing is not advantageous or is only weakly so. In other hosts, a symbiont may fail to achieve the RP phenotype seen previously (eg. Veneti *et*

al., 2012). Likewise, a beneficial phenotype conferred to one host species may not be biologically relevant for another. For example, if a novel host species is not attacked by the pathogen or parasite against which the symbiont defends, then the symbiont is not beneficial in the novel host. Intuitively, a symbiont with many phenotypes will be able to invade a broader range of host species, as there is a higher chance that at least one drive phenotype is retained in the novel host.

Heritable symbionts themselves thus represent cases where traits are laterally transferred between species within a community. This has led to them being likened to plasmids in bacteria – accessory elements that may be beneficial or parasitic, and which can cross species boundaries. They are more likely to establish in novel hosts where they carry a trait which enables invasion of the novel host – and the more traits carried, the more likely it is for the strain to establish. (See also: DOI: 10.1002/9780470015902.a0000468.pub2)

Genetic origins of 'Jekyll and Hyde' Symbioses

'Jekyll and Hyde' symbioses may evolve when a symbiont with one trait (e.g. natural enemy resistance) acquires a second trait (e.g. male-killing). The genetic basis of this acquisition is likely to commonly involve horizontal gene transfer. Co-infection, where a single host individual carries more than one symbiont strain/species, is common. Further, the intracellular nature of many uniparentally inherited symbionts put these co-existing bacteria in particularly close proximity to other intracellular symbionts. This proximity, alongside the promiscuity of microbial genomes with respect to acquiring genes from other microbes through transduction, transformation and conjugation, creates a microenvironment for transfer of traits between symbionts.

This process may explain the presence of obligate mutualist strains of *Wolbachia*, nested within a clade of facultative symbionts that are largely reproductive parasites (Nikoh *et al.*, 2014). In bedbugs, *Wolbachia* has acquired a B vitamin synthesis operon from another bacterium, and this vitamin synthesis allows the persistence of the bedbug on its B vitamin deficient blood diet (Hosokawa *et al.*, 2010). Mechanistically, bacteriophage are likely particularly important means through which genes and traits are shuttled. For instance, *Hamiltonella defensa* is a facultative mutualist that relies on an APSE

bacteriophage-encoding toxin homolog to protect its aphid host against wasp attack. This bacteriophage has been exchanged through horizontal transfer with *Arsenophonus*, a genus that includes male-killing strains (Duron, 2014). Thus, 'Jekyll and Hyde' strains may evolve through a symbiont with one trait acquiring a second trait from coinfecting symbionts. (See also DOI: 10.1038/npg.els.0001416, DOI: 10.1002/9780470015902.a0022835.pub2).

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Glossary

Symbiont - a general term to include all microorganisms, regardless of their effect, that are closely associated with a host organism

Reproductive parasite– a class of symbionts that manipulate the reproduction of a host to aid their own spread, classically associated with deleterious effects on host fitness

Cytoplasmic inheritance – the transmission of intracellular elements that occurs through egg cytoplasm only

Fitness – at its broadest definition, fitness is a measure of the survival and reproductive success of a biological entity

Gene Drive – the ability of a gene, microbe or other element to bypass classical inheritance laws and increase its odds of transmission to the next generation

'Jekyll and Hyde' infection - symbiosis in which a reproductive parasite also acts mutualistically

Obligate mutualist – a symbiont that is essential for host survival and reproduction

Facultative mutualist – a symbiont that confers a benefit to host, but is not essential for host survival and reproduction

Illustrations

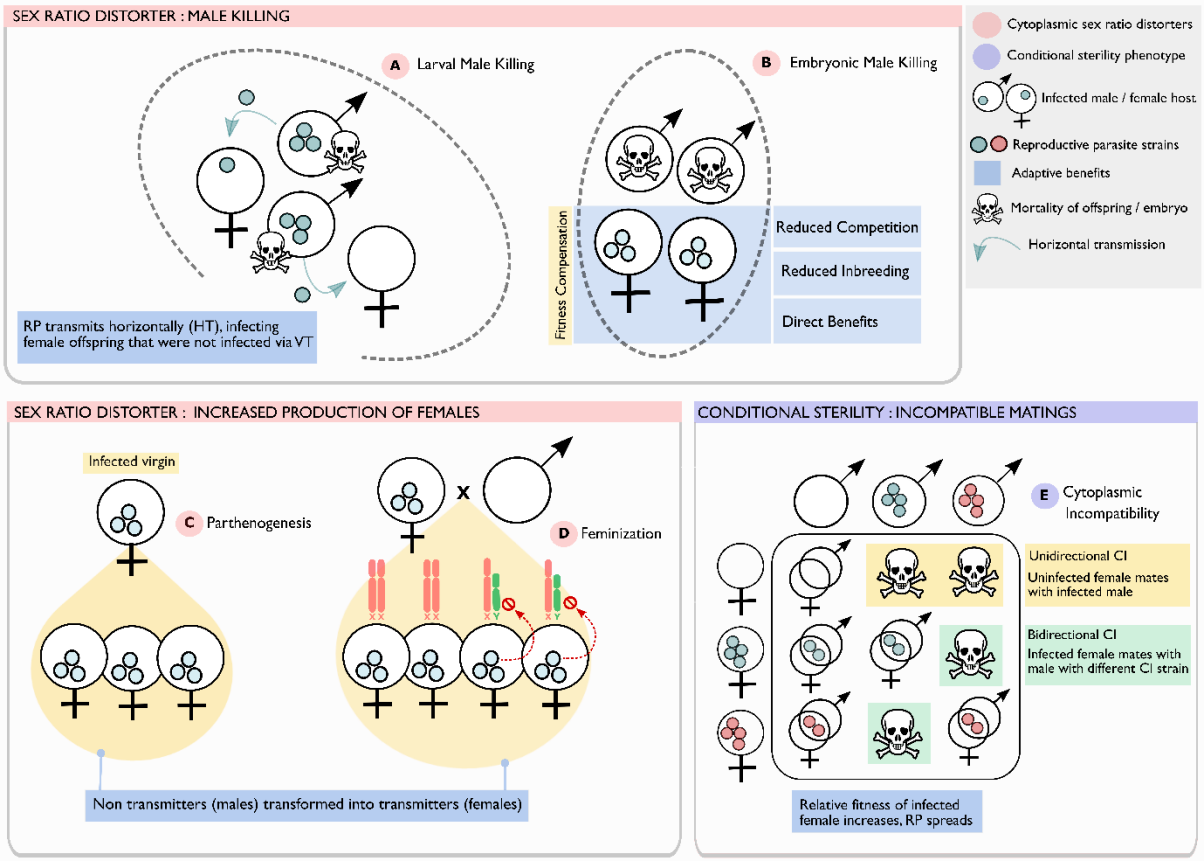

Figure 1. Common reproductive manipulation phenotypes expressed by heritable microbes are shown from A – E. All transmission of reproductive parasites (RP) is vertical unless additionally indicated and the proposed adaptive benefits of each phenotype are highlighted in blue. Phenotype (A) is expressed during the host larval stage, killing males and allowing horizontal transmission of the RP to female larvae. (B) shows the differential fate of male and female embryos under embryonic male killing. Infected virgin hosts reproduce via parthenogenesis to produce all female infected broods (C). For phenotype (D) mated infected females produce male and female offspring, but genetic males are converted to functional females. RPs produce mating incompatibilities in (E) for female hosts that are uninfected or carry a different strain, two types are shown.

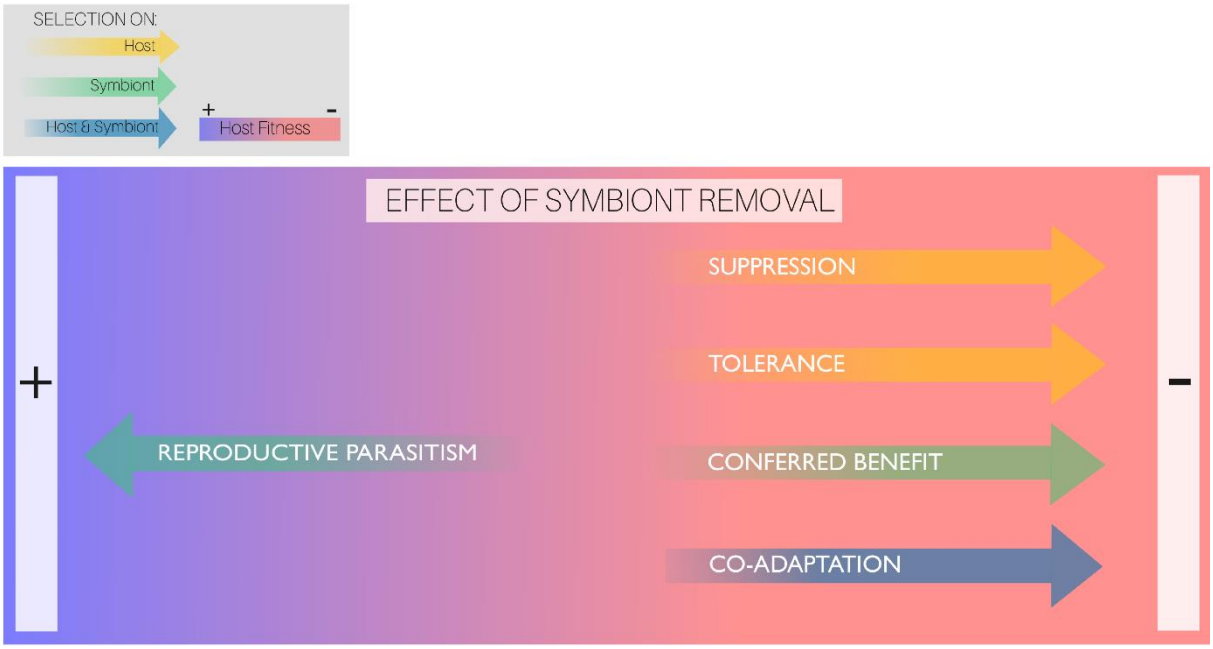

Figure 2. Possible selection pressures acting on host-symbiont interactions and the effect of symbiont removal on host fitness. Selection acts upon different members of the symbiosis (indicated by coloured arrows: host = yellow, symbiont = green, host & symbiont = blue), leading to the evolution of different situations (arrow terms). In the case of reproductive parasitism, removal of the symbiont will have a positive effect on host fitness (blue area). When other situations have evolved, to mitigate the costs of infection or confer a benefit, then removal of the symbiont can have negative consequences for host fitness (red area).

Table 1. . The mechanistic basis of reproductive manipulation phenotypes

RP Phenotype	Proposed Mechanism[1]	Genetic Basis	Heritable Microbe	References
Larval (late) male killing	Extensive replication of RP in fat body causes death of larvae during fourth larval instar	Unknown	Microspora, unnamed RNA virus	(Nakanishi *et al.*, 2008)
Embryonic (early) male killing	1 - Apoptosis in male embryos & neural malformation 2 - Interference with splicing of *doublesex*	*spaid*[2]	*Wolbachia*, *Rickettsia*, *Spiroplasma*, *Flavobacteria*, *Arsenophonus*	(Harumoto and Lemaitre, 2018; Fukui *et al.*, 2015)
Parthenogenesis Induction	1 - Feminization of haploid eggs 2 - Chromosome duplication in egg after meiosis 3 - Eggs produced by mitosis	Unknown	*Wolbachia*, *Rickettsia*, *Cardinium*	(Stouthamer and Huigens, 2003)
Feminization	1 - Prevention of androgenic gland differentiation 2 - Interference with male DNA methylation	Unknown	*Wolbachia*, *Cardinium*, *Microspora*	(Cordaux *et al.*, 2011)
Cytoplasmic incompatibility	Paternal chromosome fails to condense and mitotic disruption ensues.	*cifA*, *cifB*[3] *cidA*, *cidB*[4]	*Wolbachia*, *Cardinium*	(Beckmann *et al.*, 2017; Le Page *et al.*, 2017)

[1]Microbes may achieve a manipulation phenotype via a number of different mechanisms, if multiple mechanisms are known these are numbered. The genes involved are given, if known, but represent only a subset of reproductive parasites and may refer only to specific strains. Heritable microbes associated with each phenotype are summarised in (Engelstädter & Hurst 2009).

[2] *Spiroplasma poulsonii* MSRO

[3]*Wolbachia* strain *w*Mel

[4] *Wolbachia* strain *w*Pip

Table 2. Examples of reproductive parasites and their effects on host biology & fitness

Manipulation Phenotype[1]	Heritable Microbe	Host	Effect on Host[2]	References
MK	*Spiroplasma* (MSRO)[3]	*Drosophila melanogaster*	Protection against parasitoid wasp attack	(Xie *et al.*, 2014)
MK	*Spiroplasma*	*Harmonia axyridis* (harlequin lady beetle)	Increased body size, reduced development time & higher potential fecundity	(Elnagdy *et al.*, 2013)
MK	*Wolbachia*	*Drosophila innubila*	Viral buffering & enhanced fecundity in nutrient deprived hosts	(Unckless and Jaenike, 2012)
Female biased sex ratio	*Rickettsia* sp. nr. *bellii*	*Bemisia tabaci* (sweet potato whitefly)	Increased development rate, number of offspring & higher survivability to adulthood.	(Himler *et al.*, 2011)
PI	*Wolbachia*	*Trichogramma pretiosum*	Increased fecundity	(Grenier *et al.*, 2002)
CI	*Wolbachia* (*w*Ri)	*Drosophila simulans*	Rapid change from negative fitness costs to 10% fecundity increase	(Weeks *et al.*, 2007)
CI	*Wolbachia* + *Spiroplasma*[4]	*Tetranychus truncates* (spider mite)	Increased fecundity & development rate	(Zhang *et al.*, 2018)
CI	*Wolbachia*	*Aedes albopictus:*	Increased fecundity & longevity	(Dobson *et al.*, 2002)
Weak CI	*Wolbachia pipientis*	*Drosophila melanogaster*	Metabolic provisioning during nutritional iron stress	(Brownlie *et al.*, 2009)
-	*Wolbachia*	*Drosophila melanogaster*	Antiviral protection	(Hedges *et al.*, 2008)

[1]Abbreviations: Male-killing (MK), Parthenogenesis Induction (PI), Cytoplasmic Incompatibility (CI), (-) Undetected/Unknown
[2]Relative to uninfected host
[3]Additive with *Wolbachia w*Mel
[4]Non MK *Spiroplasma* strain