Host–Symbiont–Pathogen–Host Interactions: Wolbachia, Vector-Transmitted Human Pathogens, and the Importance of Quantitative Models of Multipartite Coevolution

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Abstract Infectious disease has been recognized for a long time as an important evolutionary force: It created the need for and shaped the evolution of immune systems and influenced reproduction as well as behavior of many host species. Infectious agents themselves also evolve and must have adapted to host strategies to evade infection, to multiple external and internal environments, and to transmission between hosts. Given the pressure to evolve on both sides, coevolution is expected. Evolution is indeed observed when looking at either host, pathogen, or at other microorganisms directly or indirectly involved and is dependent to some degree on all species interacting. Vector-transmitted diseases with high burden to humans such as malaria and dengue fever are some of many examples where parasites evade the immune system of both mosquito and human hosts, thereby maximizing the vector's transmission and persistence. Arthropod hosts such as mosquitos may also be carriers of vertically transmitted endosymbionts, such as the *Wolbachia* bacterium, that also induce a complex modification of the arthropod's life history traits. This sort of scenario illustrates the need to consider ecological, multipartite, and evolutionary models—the relevance to human health, together with extensive data collection from epidemiological surveillance, provides an opportunity to expand and improve evolutionary theory.

Keywords Infectious diseases · Host–microorganism interactions · Dengue virus · *Wolbachia* · Population biology · Coevolution

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1 Host–Pathogen, Host–Symbiont, and Symbiont–Pathogen Interactions: The Underlying Concepts

Strict definitions apart, the relationship between hosts and parasites is probably almost as old as life itself. The relevance of disease for human health made it of interest already in ancient societies, much earlier than any scientific methods could be applied or were available to investigate their properties or etiology, which was attributed to spirits or other ethereal entities such as "bad air" ("mal aire," Italian words that originated the name "malaria"). With becoming sick being such a widespread and easily recognizable phenomenon, finding out why and how it happened quickly became an obvious research program, which really gained traction with the postulation of the germ theory of infectious diseases by Pasteur (1878, revisited by Absolon et al. 1970) and Koch's postulates (Koch 1880). These and other observations that disease could be transmitted from person to person, from animals, or from foul stuff such as rotting things essentially established that all microorganisms causing disease are horizontally acquired from pathogens' reservoirs—a view which may be valid to a large extent, but is by no means complete.

Horizontal transmission implies the pathogen or microorganism can be transmitted from any host carrying it to any non-carrier, while vertical transmission is more restrictive, with transmission from parent to offspring establishing a closely related tracing of host and microorganism lineages. Notwithstanding the fact that many microorganisms were known to be transmitted by different routes and could potentially compete for hosts, the modern population biology study of host–microorganism interactions, especially of infectious disease in humans, has nevertheless been generally formulated as that of the horizontal transmission of pathogens to their hosts (Keeling and Rohani 2008). The paradigm is embraced by epidemiology (Anderson and May 1979; May and Anderson 1979), as well as by the research areas concerned with quantitative mechanistic formulations of the biological process of disease transmission in populations—the theory underlying disease ecology also dubbed theoretical epidemiology—and has been an active field of research since the first work in the early twentieth century, today almost a hundred years old (Ross 1916; Smith et al. 2012).

Vertical transmission of microorganisms, on the other hand, has been less studied and formalized under adequate ecological models although symbiosis has been a research topic of interest due to its ubiquitousness (Moran 2006). Theory on vertical transmission was not greatly furthered once some early work suggested only mutualistic associations could be stably maintained (Fine 1978), while parasitic relationships required some degree of horizontal transmission for persistence of the parasite (Lipsitch et al. 1995)—a kind of relationship that could be as easily explained was probably not as interesting. More importantly, the population biology of host–symbiont interactions was considered separate and independent from that of the same host and its pathogens, that is, host–pathogen and host–symbiont ecology and evolution were treated as distinct pairs.

Symbiont–pathogen interactions, the third possible pairwise combination of three-way interaction, however, are plausible enough if one thinks about them under an ecological perspective, as two species occupying the same niche: the host. In that case, the population biology of host, symbiont, and pathogen would all be inextricably linked. A host carrying a microorganism, say a bacterium, may be more affected if infected by a virus—e.g., it has a greater total burden of parasites—or it may be more protected—the bacterium is able to occupy the place and exclude the virus or make infection more difficult. Lively et al. (2005) suggested and formalized mathematically the possibility of a vertically transmitted parasite (VTP) to become an indirect mutualist in the presence of a more virulent horizontally transmitted parasite (HTP); simulations showed that, contrary to what previous work suggested (Fine 1978; Lipsitch et al. 1995), persistence of an otherwise parasitic symbiont that could not be transmitted horizontally was indeed possible. The result blurred even further the already blurry definition of parasite/pathogen and mutualistic symbiont.

Most of these developments do not factor in variation and natural selection, although J.B.S. Haldane has proposed, as early as in the 1940s, that disease was an important evolutionary force (Haldane 1949), and important developments have been made over the last decades, such as the modeling of reciprocal interactions between evolution and ecology (Reznick 2013; Luo and Koelle 2013), the application of models to fast-mutating viruses such as HIV (Perelson 2002), and integration of population genetic frameworks into the study of transmission dynamics (Grenfell et al. 2004; Wakeley 2005; Wakeley and Sargsyan 2009).

2 Wolbachia: Manipulation, Invasion, and Evolution

2.1 Reproductive Manipulation and Invasion

Wolbachia is obligatory intracellular, maternally transmitted symbionts of the α-proteobacteria class that are present in a large number of arthropod and nematode species (insects and worms) (Hilgenboecker et al. 2008). In arthropods, Wolbachia is a facultative symbiont which can have either a parasitic or mutualistic effect on its host (Weeks et al. 2007), although examples can be found that point to a more intimate relationships (Hosokawa et al. 2010); in filarial nematodes (round worms), for example, association is not facultative but obligatory, which happens to make antibiotic treatment effective for filarial worm infections by killing the bacteria (Beeching and Gill 2014). Wolbachia is considered a striking example of manipulation of the host by the symbiont (For a review see Werren et al. 2008); because the symbiont is transmitted maternally, any phenotype manipulation that distorts the male to female ratio such as feminization, male killing, and parthenogenesis favors persistence of *Wolbachia*.

Most notably, in some species of arthropods, *Wolbachia* can induce cytoplasmic incompatibility (CI), a phenotype by which crosses of *Wolbachia*-carrying

males with non-carrying females result in little or no offspring, while that of female carriers with male non-carriers have normal viability. Because the former are crosses with non-carrying mothers (which do not result in symbiont-carrying progeny) and the latter results in carriers, CI gives *Wolbachia* carriers a selective advantage, increasing the frequency of the symbiont in the population (Fig. 1).

Cytoplasmic incompatibility is often conceptualized as a modification–rescue, or lock-and-key mechanism (Merçot and Poinsot 2009): Individuals carrying *Wolbachia* are able to induce incompatibility by modifying the sperm's cytoplasm, and, in order to produce viable progeny, the egg's cytoplasm must be able to be rescued—a compatible Wolbachia in the mother can come to the rescue. Modification and rescue can in principle be uncoupled, giving rise to different phenotypes, e.g., suicide symbionts, which are able to induce incompatibility when in the male but not resist it when in a female body. These types and their importance for persistence and evolution of the symbiont are discussed in the following section; for clarity, unless stated otherwise, when CI-inducing *Wolbachia* is mentioned here, it refers to microbes that both induce incompatibility to hosts that are not carriers and that give rise to viable offspring in an otherwise incompatible cross with a carrier.

Evolution of arthropod carriage of *Wolbachia* (at this point ignoring host, symbiont or any other coevolutionary responses to the presence of the symbiont) is conceptually equivalent to that of a mitochondrial gene—i.e., exclusively transmitted by mothers to their offspring. Using basic selection theory, Caspari and Watson (1959) described the dynamics of the frequency of (what was postulated to be) a maternally transmitted unidirectional cytoplasmic sterility factor between the Oggelshausen (*Og*) and Hamburg (*Ha*) strains of *Culex pipiens* mosquitoes: Female *Ha* X male *Og* crosses were viable, but male *Ha* X female *Og* crosses would give no progeny, all offspring dying as embryos. Still under the population genetics framework of basic selection theory, Turelli and Hoffman (1991) described the dynamics of *Wolbachia* based on the observation that in *Drosophila simulans* it induced both unidirectional incompatibility in crosses and a fecundity cost to carriers (Turelli and Hoffman 1995; Carrington et al. 2011).

A large body of theoretical work was developed around the main result that establishment or elimination of *Wolbachia* is depended on the initial frequency of

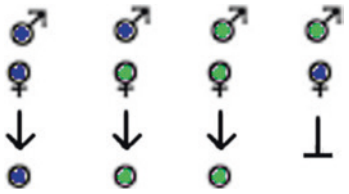

Fig. 1 Cytoplasmic incompatibility. Four crosses are possible between male and female carriers or non-carriers of *Wolbachia*. When the symbiont induces cytoplasmic incompatibility, the cross between a male carrier and a female non-carrier is sterile, or significantly reduced; therefore, carriers of *Wolbachia* have the advantage of having two out of four viable crosses against only one out of four of non-carriers

the symbiont in the population: If the initial frequency was above a given threshold, *Wolbachia* would get fixed; otherwise, it would be eliminated (Hoffman et al. 1986; Jansen et al. 2008; Turelli 2010; Barton and Turelli 2011). Under this model, the invasion threshold was found to be determined by the ratio of fecundity costs to intensity of cytoplasmic incompatibility. Additionally, other costs or *Wolbachia*-associated effects could be present and it does not follow trivially that a simple cost/advantage ratio describes the invasion threshold in those cases. In fact, taking into consideration the specific ecological processes such as birth and death rates, for instance, the threshold can be shown to be dependent on these rates (Souto-Maior et al. 2015) and that the reason they do not appear as such in some of the previous formulations is because the processes are either absent from the model or fall into a special case where they are aggregated and equivalent to fecundity (Hoffmann et al. 1990; Hancock et al. 2011).

One example of a somewhat unexpected effect associated with *Wolbachia* is antiviral protection conferred by naturally occurring strains of *Drosophila melanogaster*, described first in fruit flies challenged with Drosophila C virus and Flockhouse virus (Teixeira et al. 2008; Hedges et al. 2008). The *D. melanogaster*-derived strain *w*MelPop was adapted through passage in mosquito cell lines and transferred to *Aedes aegypti* mosquitoes; it was found to confer protection against RNA viruses causing human disease such as dengue virus (DENV) and chikungunya, as well as against *Plasmodium galinaceum*, a malaria parasite that does not infect humans (Moreira et al. 2009). *Wolbachia* of the *w*MelPop strain transinfected to mosquitoes imposed both fecundity and longevity costs, while on the other hand, protecting mosquitoes against mosquito pathogens, the dynamics of symbiont carriage is more complicated in that case, and population genetic models fall short of describing it.

Picking up on the tripartite interaction framework (Lively et al. 2005; Jones et al. 2007), ecological models were used to describe the effect of protection in *Wolbachia* invasion (Fenton et al. 2011), showing that protection against a virulent pathogen combined to CI facilitated invasion of Wolbachia imposing a fecundity cost and having imperfect transmission from mother to offspring. Simulations that factored in age structure showed that timing of introductions affected the probability of invasion, so planned releases should consider timing (single versus multiple releases), because the threshold alone no longer predicted success of the invasion (Hancock et al. 2011). Further theoretical work also demonstrated that the threshold of invasion could be analytically calculated in the presence of fecundity, longevity costs, and protection against pathogens, that each *Wolbachia*-associated effect had a weight in the threshold proportional to importance of the ecological process affected, and that heterogeneity in these effects could impact invasion (Souto-Maior et al. 2015). The usual culprits of dismantling predictions of simple, deterministic models, spatial structure and stochasticity can also affect the probability of invasion (Hancock and Godfray 2012; Barton and Turelli 2011; Jansen et al. 2008). Therefore, unlike some early results suggested, not all costs affect invasion in the same way; ecology and age structure affect establishment of *Wolbachia*; and spatial spread does not follow trivially from local invasion.

Despite the vast theoretical work developed around a single symbiont, and most notably one particular reproduction manipulation phenotype out of the many observed, obtaining estimates for all known relevant parameters is not trivial, and more elaborate frameworks may be necessary to estimate some of them (Pessoa et al. 2014), or quantify environmental effects such as pathogen burden, which is likely to depend on a multitude of microbial species (Calzolari et al. 2012). Furthermore, the models discussed so far concern only evolution of the host insofar as there is selection to carry or not carry the symbiont—i.e., the symbiont is a fixed set of genes inherited separately from host nuclear genes, and it may be or not be advantageous to have those genes. Host and symbiont genes are of course not fixed, but exhibit heritable variation among individuals. Although not formalized into a quantitative framework, there are arguments that there should be host responses to manipulation by the symbionts (Merçot and Poinsot 2009; Vavre and Charlat 2012; Tortosa et al. 2010). However, the closest to any kind of formalization of the a real-world situation came from the need to assess the impact of *Wolbachia* on DENV transmission—since releases already took place in different parts of the world and more were scheduled to happen in 2014 in more countries—and is in the form of an "evolutionary forecast" (Bull and Turelli 2013). Likely scenarios were forwarded with preliminary predictions that need to be further studied to better understand the consequences of such a provoked human intervention in a complex environment.

2.2 Symbiont Evolution

Besides carrying or not carrying *Wolbachia*, evolution of host nuclear genes and of *Wolbachia* itself is expected and, as mentioned above, the host may respond to the effects of the symbiont, which in turn may tweak its effects to persist, all that conditional on variants being present are selected for the advantageous traits.

Turelli (1994) formalized the evolution of incompatibility inducing microbes into a mathematical model and concluded that after being driven in by cytoplasmic incompatibility, there would no longer be any selection for the trait (as long as any microbe variants present were mutually compatible) and that rather, selection would tend to attenuate any costly effects of these symbionts. Merçot and Poinsot (2009) reasoned that, in that case, and considering cytoplasmic incompatibility as a modification–rescue phenotype and with no pressure to maintain CI, selection or random genetic drift could cause variants not inducing the phenotype to be fixed in the population instead. As soon as there were no CI-inducing individuals, a rescue-only variant would not have any pressure to maintain that trait either, and the result could be a symbiont carrier neither inducing nor resisting CI. Although this is conceptual and not quantitative reasoning, the expected intermediate variants of *Wolbachia* have been observed in nature (Merçot and Poinsot 1998; Bourtzis et al. 1998; Charlat et al. 2003; Zabalou et al. 2004).

Additional population structure could favor some selection for CI persistence, although it is thought not to be as strong as selection for increased fecundity (i.e., decreased fecundity cost), so persistence of the CI phenotype in many species is not entirely accounted for (Haygood and Turelli 2009). *Wolbachia* in those cases would be "passengers" that were able to squeeze in through manipulation, differently from mutualists, which always have selection favor their persistence, or their status in nematodes, where the association is obligatory for host survival; in the latter cases, the symbiont would actually be a "resident" (Merçot and Poinsot, 2009), propagating and persisting together with its host as one unit.

Uni- and bidirectional cytoplasmic incompatibility are striking, conspicuous manipulative phenotypes; explaining its emergence and persistence is of interest from the point of view of the evolution of complex traits, of manipulation in symbioses, and their possible role in speciation (Faria and Sucena 2014). In a much more straightforward prediction, if fecundity is heritable and variants with high fecundity are present, the trait is always expected to evolve, because by definition, they have increased fitness: They leave more offspring and increase in frequency—as discussed above, they may even offset stronger or more striking phenotypes such as CI in the long run (Haygood and Turelli 2009). In *D. melanogaster,* the intensity of CI (percentage of offspring killed in incompatible crosses) is quite weak (Hoffmann et al. 1990); in the sister species *D. simulans*, CI is stronger, and still the effect of *Wolbachia* on fecundity was observed to evolve from costly to beneficial: The association of *Wolbachia* and *Drosophila* evolved from a parasitic to a mutualistic one (Weeks et al. 2007).

Less obvious is the impact of other traits induced by symbionts, such as reduction in life span (or longevity cost) or protection against pathogens; even though both are supposed to have a positive effect, these depend on exactly how they increase fitness (i.e., how they translate into a selection coefficient), e.g., a longevity cost may have no impact if it causes individuals to die only after laying all eggs it could. Protection against pathogens depends on a burden of pathogens actually being present. Also, trade-offs between traits may cause selection of one but not other trait, i.e., individuals with higher resistance against pathogens may have lower fecundity. There is evidence, for instance, of a global replacement of more protecting strains by others that would protect less, but have increased longevity (Riegler et al. 2005; Chrostek et al. 2013).

Extending what earlier population genetics purported to do into the evolution of a symbiont inducing multiple changes in the host life history is a challenge, especially when the host is likely to coevolve in response to the new association: an arthropod host with a specific genetic background will evolve either while carrying *Wolbachia* or without it. The genes can arrive at a host through various combinations of crosses: A mother carrying *Wolbachia* may give rise to some offspring without *Wolbachia* (due to imperfect transmission), and a father without *Wolbachia* in a cross with a female carrier will see its offspring emerge as carriers. Evolution of *Wolbachia* will only happen inside its host, but will also be conditioned on host genetic background and host–symbiont feedbacks. Disentangling this sort of confusion is necessary to minimally describe coevolution.

Fuzzy as it may seem, though, this still only describes host–symbiont coevolution, ignoring other microorganisms and longer term genome interactions that may occur over greater evolutionary timescales. Despite not being entirely within the scope of theoretical population genetics, some observations of natural populations can hint at the evolution of host and symbionts in the presence of other microorganisms.

3 Host–Microorganism Genome Interactions and Long-Term Relationships

The origin of organelles such as mitochondria is generally accepted to be of endosymbiotic nature. Many variations of the same organelle theme suggest that the host nuclear genome incorporated many of the genes originally in these free-living organisms turned resident machinery. Although it is different, transfer of genetic material is postulated to have happened from *Wolbachia* to its hosts (Nikoh et al. 2008).

In *D. melanogaster*, the association with *w*Mel was estimated to have the symbiont's most recent common ancestor at around 8000 years ago, with strong association between the phylogenetic relationship of the symbiont and mitochondria, therefore forwarding the idea that the currently observed patterns are best explained by a single acquisition and subsequent loss of *Wolbachia* in some lineages afterward (Richardson et al. 2012). Indeed, large chunks of *Wolbachia* genes have been found to be incorporated into the nuclear genome of some insect and nematode species and are found to be transcribed (Hotopp et al. 2007; Nikoh et al. 2008); as with mitochondria, it is unclear to which extent nuclear genes are functional and could take over the functions performed by the symbiont—thereby rendering it useless—but any such events could greatly affect coevolution of host and symbiont, possibly shifting the balance in any existing marriage conflicts. Lateral gene transfers and host jumps (symbiont horizontal transmission) are believed to be rare and are therefore not expected to be observed in shorter timescales; nevertheless, artificially introducing a *D. melanogaster*-derived strain of *Wolbachia* *w*Mel into a new species, such as *A. aegypti*, may see many features of a novel association, which may be compared to what is observed in older couples.

Some statements about old and new associations need to be reconciled, but it is not always easy to do so; one of the most difficult to make sense of is "*Wolbachia* confers antiviral protection to its host." Once more strict assertions are made, what can really be said is something less general: *w*Mel, the *D. melanogaster*-derived *Wolbachia* strain, confers antiviral protection to its *Drosophila* species (Teixeira et al. 2008; Martinez et al. 2014), and indeed, the same strain (and the related *w*MelPop) confers protection against DENV in mosquitoes *A. aegypti* and *Aedes albopictus* (Moreira et al. 2009; Walker et al. 2011; Blagrove et al. 2012). However, *A. albopictus* is naturally a carrier of not only one, but two different strains of Wolbachia (*w*AlbA and *w*AlbB), and is a competent vector of DENV:

Although usually considered an inferior vector than *A. aegypti*, in the absence of the sister species, it can efficiently transmit disease (Lambrechts et al. 2010). So the endogenous strain in one old host, *D. melanogaster*, confers protection, but in another, *A. albopictus*, it does not; while it can be argued that these are strain-specific properties or that the density of symbiont can explain those properties (Lu et al. 2012), the possibility of these observations being the result of a coevolutionary process involving both host and *Wolbachia* cannot be discarded. This is a concern particular for *Wolbachia*-based interventions aimed at human health, like that of provoked invasions of *Wolbachia*-carrying *A. aegypti* to block DENV transmission.

For a disease control intervention based on *Wolbachia*, the ideal scenario is that of a successful introduction of a reproductively costly, life-shortening, DENV-blocking strain that could overall reduce transmission to levels below the epidemic threshold, i.e., no sustained transmission could be maintained. This could also be achieved with a DENV-blocking strain with high protection (measuring absolute protection is tricky, but see Gomes et al. 2014; Pessoa et al. 2014). If no evolution was expected, continuous DENV transmission could be eliminated in areas where *A. aegypti* is the only or main vector, which is the case in urban areas; because the *w*Mel–*Aedes* association is not a natural, and therefore unlikely to be an evolutionary stable one, there is a reason to expect immediate response of the host, if it is evolutionarily perceived as an aggression and if there is variation in the population that can be selected to counter these effects. Some speculation could be done on how the mosquitoes could be unhappy at their new guests, but actually identifying the tools to respond is more complicated, and experiments rely on artificially selecting insects for many generations (Martins et al. 2013).

Additional insight can be gained by looking at different species with different kinds and times of associations; *w*Mel in *A. aegypti*, for instance, is seen to increase expression of immune genes (Bian et al. 2010). Even though this is not observed in its original *Drosophila* host even with foreign symbionts (Chrostek et al. 2014), it raises the question of whether the antiviral protection in mosquitoes is a result of priming the immune system (Ye et al. 2013) with a new, strange thing and that the immune upregulation could go away once the host got adapted to its new passenger—having over-activated immunity could also explain some fitness costs in terms of collateral damage inflicted by immune cells that normally would not be active, but are out to kill something (Schneider 2011).

That would also be consistent with *A. albopictus* not having noticeable protection despite naturally carrying two *Wolbachia* strains, but being more protected when carrying the newly transinfected *w*Mel. Punctual observations such as these comparative analyses, however, can suggest some hypothesis and rule out a few predictions, but cannot explain or predict a quantitative outcome such as elimination of endemic transmission, and stability of the strategy in the long run.

Existing long-term relationships of arthropods and *Wolbachia* can suggest possible outcomes of new introductions of the symbiont into a new host; however, because host jumps occur, naturally occurring associations are not guaranteed to be old, and estimates of the time since the symbiosis exists are necessary to

assess whether enough time has elapsed for any kind of equilibrium to be attained (and even so there is still no guarantee that it has). Ancient symbioses may have a more complicated interpretation if the partners exchanged genes. Many of those aspects are either beyond the scope of population genetics or cannot be analyzed under classic population genetics, or are too hard to formalize at this point, and even if it was done, measuring and characterizing the existing variation requires a heroic effort to measure a single (albeit possibly one that could be very important) parameter. Like the more general retrospective phylogenetic analyses, prediction in this situation is more akin to some sort of weather forecast than the rigorous hypothesis population genetics usually forwards.

4 Dengue Virus: The Case for Evolutionary Medicine and Evolving Vaccines

DENV belongs to the flaviviruses, a single-stranded RNA virus family; it can alternatively be more loosely classified as an arbovirus, or viruses that are borne by arthropods. It comprises four related serotypes that are moderately close (uncreatively named DENV-1 through 4), sharing a little over 65 % of genetic sequence similarity (Guzman et al. 2010). The viral structural and non-structural genes, general characteristics, as well as the clinical disease caused by any of the serotypes are similar, which includes undifferentiated fever, joint pain, strong headaches, rash, as well as usually mild bleeding manifestations; unique characteristics of any one serotype are unknown or unresolved (Halstead 2007a). The disease is transmitted to humans by female mosquitoes of the genus *Aedes*, the transmission cycle being maintained by continual transmission from a human to an arthropod host, but a sylvatic transmission cycle can be maintained between monkey and other mosquito species normally not interacting with humans (Vasilakis et al. 2011).

Almost 3 billion people live in areas with the risk of DENV transmission, and it is estimated that there are some 300 million cases every year (Bhatt et al. 2013); there is no specific treatment for the disease, and management consists of supportive treatment of the symptoms. Uncomplicated cases resolve themselves, but more severe manifestations of dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS) can have a case fatality rate of 50 % if untreated due to hemorrhagic manifestations. DHF/DSS cases are associated with secondary DENV infections, but have been observed in primary infections, particularly in children (Halstead 2007a, b), and where factors affecting coagulation are involved—which is why the use of salicylic acid or other drugs affecting blood clotting is not recommended to relieve symptoms. It has been argued that because of the increased risk associated with secondary infections, an effective vaccine should confer high protection against all four serotypes; clinical trials of a vaccine have recently been conducted, with mixed results (Costa et al. 2014). Given the difficulties, most control measures focus on the management of the vector population.

After an infectious mosquito bite, there is an incubation period which, although normally not possible to measure directly or with high precision, is generally accepted to be somewhere between the 3–14 days interval (WHO); if infection is successful, disease follows and is normally self-limiting—at least in uncomplicated cases—after about 7 days, period during which the human host is most likely to transmit to any uninfected mosquitoes that feed on his or her blood (Nguyet et al. 2013). After an infectious blood meal, the female mosquito may develop disease and become infectious after about 10–14 days (Halstead 2007b); infected mosquitoes are believed not to recover from DENV, and the disease halves the life span of the mosquito (Maciel-de-Freitas 2011).

The DENV serotypes are antigenically distinct—infection with one serotype confers lifelong protection against a second infection with the same serotype, but infection by any of the other three serotypes is still possible—it is still a matter of discussion whether secondary infection is less, equally, or more likely that primary infection, or if instead a temporary cross-immunity, plays any role in DENV epidemiology (Johansson et al. 2011). The chain of transmission is shown in Fig. 2.

The processes just described can be formalized into a mathematical model (or alternative models) of DENV transmission (Nishiura 2006), with the observed waiting times of each process being converted into rates or probabilities that each event happens (Johansson et al. 2011), i.e., the model parameters. Although some of these parameters can only be measured imprecisely, indirectly, or not at

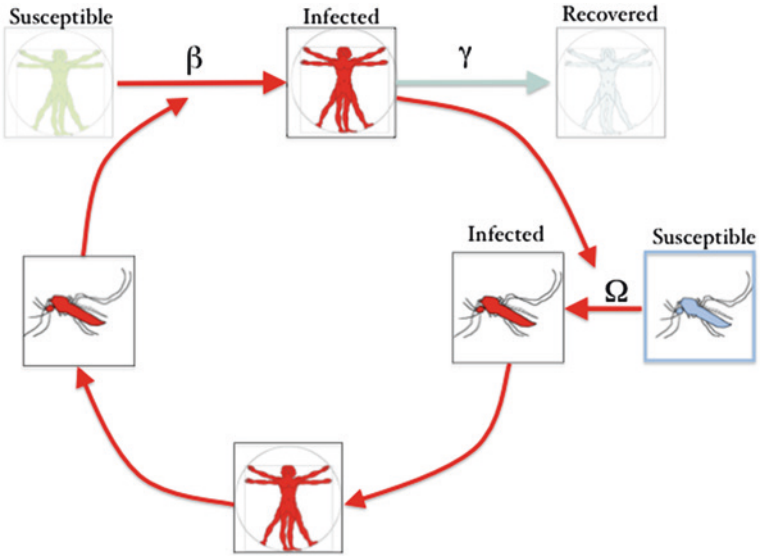

Fig. 2 Dengue virus transmission cycle. Infected mosquitoes bite and infect susceptible humans with probability β, which later recover at rate γ. Infected humans bitten by a susceptible mosquito transmit disease with probability Ω. The continual cycle maintains endemic transmission if the transmission rates are high enough

all, building such a model allows for simulating disease transmission within what is thought to be a reasonable range of parameters; the patterns can then be analyzed and compared to the observed disease incidence (Keeling and Rohani 2008). Despite the series of assumptions and simplifications that are needed to come up with what is already a quite complex model (Gunawardena 2014), some general features of interest arise, like the sustained multiannual oscillations in the number of cases that are a result of the interaction of two or more serotypes (Esteva and Vargas 1999; Luz et al. 2003; Wearing and Rohani 2006; Nagao and Koelle 2008; WHO-VMI Dengue Vaccine Modeling Group 2012).

Multiannual cycles are observed in epidemics of DENV in places where more than one serotype was known to have circulated; nevertheless, whether the cycles are a result of heterologous serotype temporary cross-immunity, permanent cross-protection or cross-enhancement cannot be straightforwardly resolved by simulation alone (Adams et al. 2006; Cummings et al. 2005), since the model outputs are similar for either mechanism. Simulation can therefore only find whether the proposed mechanism can broadly generate the observed behavior. Despite all the complexity involved, it is worth reminding that these models assume all population traits are fixed, i.e., there is no heritable phenotypic variation in any of the populations and therefore no evolution.

On the evolution side, *Aedes* mosquitoes have been studied using the modern synthesis–era theory of population genetics, with measures of expected genetic differentiation of species such as F_{IS} and F_{ST}, (e.g., Lourenço-de-Oliveira et al. 2004, Bracco et al. 2007), which do not consider any specific models of mosquito population dynamics and do not explicitly account for any selective pressures arising from pathogens or symbionts. The question answered by this approach is mainly "is the genetic structure of two (or more) populations different from one another?" but not "how much does having (or not having) the presence of pathogens or symbionts (possibly at varying levels) affect genetic structure?" These measures are also not easily connected to the selection of any specific traits or population processes and only indirectly answer questions about the processes that generated the current diversity.

Evolution of DENV, in turn, is studied mostly via phylogenetics (Miagostovich et al. 2006; de Castro et al. 2013; Faria et al. 2013; de Araújo et al. 2012), trying to make sense of clade replacements in terms of selection in favor of a specific genotype; increased incidence of an outbreak is associated with a genotype being used as a proxy for greater fitness. Despite having a mutation model implicit in the phylogenetic clustering algorithms, results are essentially decoupled from any mechanistic model of disease transmission, and in most cases, results cannot be used to interpret the patterns of disease transmission quantitatively (but see Mondini et al. 2009; Rasmussen et al. 2014 for work that begins pushing in that direction in the context of neutral evolution).

Observations such as the low in vitro replication rate (which could be a component of virus fitness) of the American DENV-2 are nevertheless inconsistent

with the large outbreaks observed for the strain, for which newly arisen virulence mutation markers could not be identified (Halstead 2007a). Some research has also attempted to make sense of increased fitness of a clade in the context of immunity conferred by other serotypes, where a reasonably large amount of serological a genetic data was available from patients (OhAinle et al. 2011); however, the number of hypotheses, correlations, and comparisons is quite large—interpretation of the results can be cumbersome, and statistical power to identify large significant effects is probably low.

Describing phenotypic evolution of DENV in terms of mechanistic processes in disease transmission requires the heritable genetic variation in the population of viruses (and, if coevolution is to be modeled, also in mosquitoes and humans) to be quantified; it is not trivial to find whether variation is present for, for instance, insect resistance against pathogens (Martins et al. 2013) and much less for all relevant parameters in the transmission model, so a mechanistic description of phenotypic evolution that is realistic would require a large amount of prior information. Furthermore, selective pressures acting on the virus are likely to be very different whether inside the human rather than the mosquito host, and evolution then depends on a balance of what is selected in each case, as well as on the rate of transmission between hosts, which is also closely related to viral fitness.

Neutral molecular evolution, on the other hand, has recently had reasonable success in being incorporated into parametric models (Volz 2012; Volz et al. 2013); as usual, several assumptions go into making that possible, such as assuming infection consists of a single viral sequence, and not a dynamic, mutating population of viruses—so even the observation that infection consists of a polymorphic viral population has yet to be developed (but see Gordo and Campos 2007; Gordo et al. 2009).

Whether evolution of the human population feeds back (interacts reciprocally) into evolution of DENV is not clear, since the disease does not seem to impose a high mortality burden; for mosquitoes, despite the high virulence (Maciel-de-Freitas et al. 2011), it is believed that less than 1 % of mosquitoes would be infected with DENV at any one time, so that the virus could not affect much evolution of the vector—quantification of these predictions is not straightforward.

Models were built to predict the evolution of the virus under interventions aimed at controlling the disease (Medlock et al. 2009); the use of *Wolbachia* to introduce resistance into and manipulate the life history of the mosquito population has to be analyzed under a similar light, although the interactions are much more complex in the latter case. As mentioned above, the tripartite interaction could induce feedback in the evolution of host, symbiont, and pathogen and affect the immunity profile of the human population if transmission was halted or reduced to low levels. While difficult to formalize the multiple interactions, mathematical models can help analyze possible outcomes, when intuition alone is at loss given the number of subpopulations and parameters.

5 Using Human Disease as a Model to Study Evolution: Difficulties in the Description and Prediction of Host–Microorganism Associations and "Evolutionary Forecasts"

Evolutionary theory has come a long way from the Darwinian proposal of natural selection; theoretical population genetics was in the forefront of formalizing its concepts, thus consolidating the modern synthesis, proposing new ones such as neutral evolution, and providing testable quantitative hypotheses. Mathematical modeling of ecological and epidemiological dynamics, as first introduced by Ross (1916), has also come a long way, and computer simulation of huge, complex systems that are mathematically intractable has more or less recently become possible. Statistical inference is still a somewhat limiting factor, although Bayesian methods started to be more widely applied and have recently used the power of computers to push likelihood-based methods beyond what the more traditional least-squares fitting methods can achieve (Myung 2003; Lavine 1999).

More limiting is our ability to develop models that, at the same time, capture enough detail of the processes of interest and are tractable enough to be analyzed and explored and amenable to statistical inference. Currently, a great challenge is to use the mathematical and statistical tools to expand the description of biological systems and obtain high-resolution information about evolutionary processes; in the processes, some classic results may be improved, while others will be refuted, which will help overturn current consensus and dogmas that may be in the way of scientific progress.

Given the relevance of human pathogens, exploring and trying to predict the consequences of medical interventions is indeed a matter of life and death. Some of the theory has been put in place to develop and further the field of evolutionary medicine and epidemiology (Price 1970; Gandon and Day 2007), and there have been calls to make good use of the models (Vavre and Charlat 2012), but a full-fledged framework is still lacking that is applicable to real epidemics and interventions such as vaccination programs and more experimental strategies such as the use of *Wolbachia* (which conceptually is equivalent to vaccinating mosquitoes).

Vavre and Charlat (2012) argue for extending the study of the dynamics of the host–*Wolbachia*–pathogen "*menage a trois,*" and the coevolution of pathogen virulence and prevalence together with symbiont-mediated protection—they also note that the presence of CI may release selective pressure for high protection—an important, unexplored question. Not mentioned is that selection for CI can be relaxed in the absence of non-carriers, as discussed above.

Bull and Turelli (2013) propose and discuss plausible scenarios for the post-intervention of at least three strategies involving the release of *Wolbachia*-carrying mosquitoes: life-shortening *Wolbachia*, CI-based population suppression, and DENV-blocking *Wolbachia*. They argue that in the case of life-shortening, there would be intense selection for decreasing the effect in both host and symbiont, since life-shortening decreases fitness of both, while the virus would see more

(but may be not a lot more, since there it should already be strong) selection for a reduced incubation period that allowed it to be transmitted sooner. For suppression, there would be strong selection to escape CI or avoid mating with carriers; in *D. simulans,* there seems to be little or no genetic variation for these traits, and if it is also true for *A. aegypti,* short-term success is likely, and over the long run, selection should overturn the results. In the case of blocking, the situation is the most complicated; there is a conflict among the partners, with the host enjoying antiviral protection, no direct selection on *Wolbachia*, and the virus being selected to escape. Information on standing genetic variation for those traits is essentially unknown. The authors also acknowledge that extending the reasoning to a model-based inference is unreliable due to the multitude of parameters and potential trade-offs.

The difficulties that arise in trying to predict evolution stem from the fact that it requires formalizing a model and its parameters, specifying the heritable variation for each of them (or at least the important ones or those expected to evolve), as well as characterizing trade-offs between any two or more of them—not a straightforward task at all. More worrying, a model-based framework to estimate pre- and post-intervention parameters is essentially absent; evaluation of interventions is done through randomized trials, and general conclusions cannot be extracted from them beyond whether the intervention has any success in that particular place and time (but see Gomes et al. 2014). Research on this interface could go many ways, with each specific research area informing the others: evolution, ecology, medicine, epidemiology, and modeling of complex systems. A summary of the interactions and illustrative evolutionary outcomes of host, a pathogen, and a symbiont is shown in Fig. 3.

5.1 Multipartite Interactions

Tripartite and multipartite interactions are, however, not an exclusivity of host–microorganism interactions; one can picture that a carnivorous animal, an herbivorous one, and one (or more) species of plant are coevolving in response to forces exerted directly or indirectly by each of the players that may decrease the fitness (most easily depicted by the carnivore killing and eating the herbivore, thereby reducing the fitness of the latter to zero after the event).

Nevertheless, Wright–Fisher population models and simple extensions thereof (models with constant population size or constant intervals) have been used for a long time to obtain estimates of population parameters of animal species (Rosenberg and Nordborg 2002; Quéméré et al. 2012; Heller et al. 2013); there is no a priori reason why host–microorganism interactions should be any different, and yet epidemiologists are usually not satisfied with the direct application of theoretical population genetics to disease transmission and host–microorganism interactions in general (Volz et al. 2013). There are a few reasons why that is so, and these probably offer new opportunities to population genetics and possibly to evolutionary theory alike.

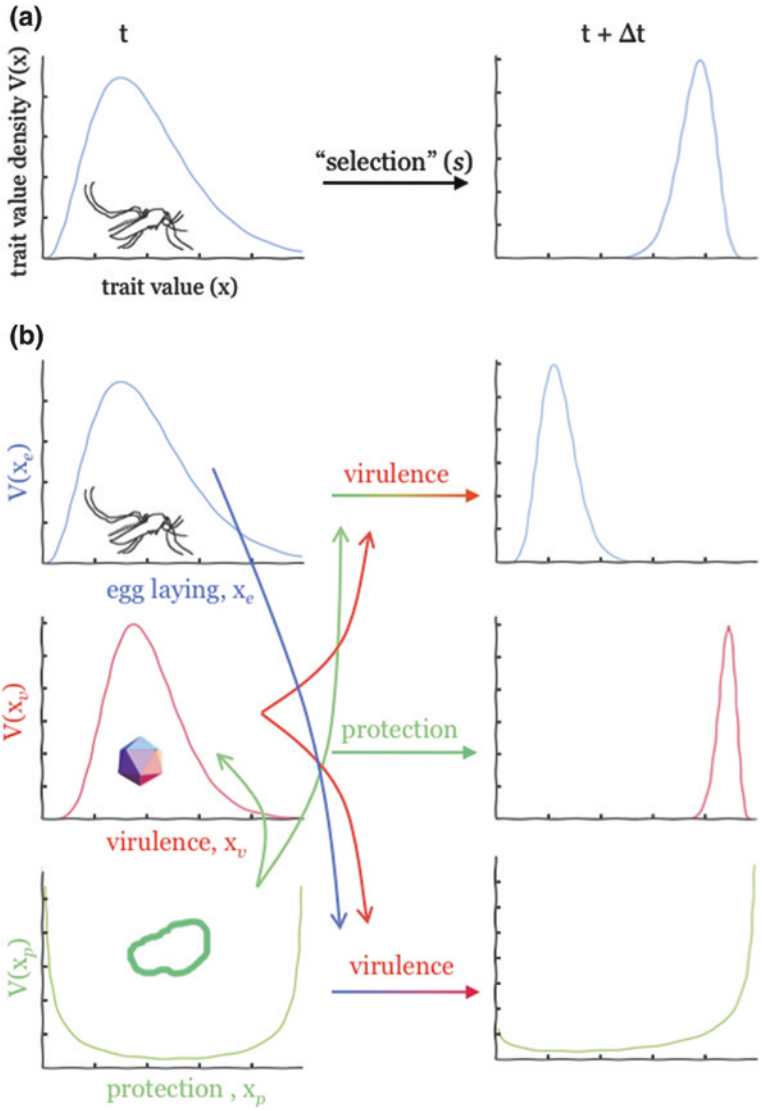

Fig. 3 In more traditional population genetics models, a trait may be thought to be under a selective pressure, broadly speaking, that would, for instance, select for higher values of the trait. The distribution of the trait after a time Δt is the function of the phenotypic variation, $V(x)$, and the selective pressures, s (A). In models of interactions of host, pathogen, and symbiont, the selective pressures arise from the interaction of the different species on each other, and therefore, all of the trait distributions after a time Δt are functions of all original distributions ($V(x_e)$, $V(x_v)$, $V(x_p)$) as well as of the different selective pressures (virulence, protection, etc.), which may all vary nonlinearly over time (as opposed to being a constant "s" coefficient)

First, the theoretical framework to describe the population dynamics of pathogen infections represents an entire new field of research, and theoretical epidemiologists are probably not ready to abandon it entirely in favor of simpler and even naïve models, even if consolidated as useful tools in population genetics. Second, the timescale and resolution investigated for animal systems are much larger, and the fine-grained population dynamics are secondary, with major features being more important because they are able to give insight into unobservable processes removed so far back in the past that any information is very valuable nonetheless. Third, and putting the second and first together, disease ecology and epidemiology aim to answer specific questions concerning the distribution and risk of disease, which are not necessarily the same questions of interest from animals or plants, e.g., for conservation purposes, it is of interest to know whether an animal population had its diversity affected by a bottleneck or fragmentation of its habitat (Heller et al. 2013), while for disease bottlenecks are part of everyday life, and their high mutation rates help them make up for the diversity lost after a population crash (also known as elimination or disease control). Additionally, important classic metrics of population genetics often do not have an obvious meaning for epidemiologists; effective number of infections (Frost and Volz 2010), the epidemiological equivalent to the effective population size, is not immediately interpretable or useful for medical purposes.

On the flip side, disease ecology allows observation of some (in animal ecology) unobservables. While the number of animals is generally unavailable, or requiring extensive efforts to obtain a vague idea of its census population, a reasonable-though-imperfect count of the number of infections—the size of the pathogen population—is not breaking news. The number of disease cases is often public and obtained through health surveillance systems designed specifically to record not only incidence/prevalence of disease, but increasingly also patient serological information and pathogen strain/genotype (SINAN). Therefore, inference from sequence data can in principle be directly compared to the observed population dynamics, i.e., estimates such as effective population size can be compared to actual population size to see whether the quantity actually gives information about the real population. Because of the short timescale and high mutation rates, this kind of inference can be made for many "replicates" of the natural experiment. Also, because of the simplicity of viral pathogens, arguably with little or no recombination and strictly asexual reproduction, they best approximate the common assumptions of population genetics and their genomes are closest to genetic recording machines (although for many reasons not nearly as much as researchers think or would like them to).

6 Concluding Remarks

While it requires working on the interface between ecology; theoretical population genetics; medicine and epidemiology; and biomathematics and statistics, studying disease as an evolving system can help answer interesting questions to the

different research areas. The difficulties in mathematical and statistical modeling apply throughout; however, the ecological framework is in place, researchers start to pick up where classical population genetics left off, and furthermore, epidemiological data are available, but yet to be fully incorporated to the analyses. A new movement to synthesize these parallel efforts, as well as hard work on the methods, can help extend evolutionary theory further.

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Glossary

Artificial selection Consists of creating conditions (usually in a laboratory setting) that favor certain variants provided they exist in the previous populations—to be more represented in the following generations, e.g., infecting flies with a virulent pathogen will cause resistant flies to increase in frequency. Artificial selection is useful for detecting and quantifying variation for certain traits

Bayesian statistics One of the two main statistical philosophies, as opposed to frequentist statistics, based on the original work of Bayes, requires using (or assuming to the best of knowledge lack of) prior knowledge as input and dealing with uncertainty in the parameter estimates

Cross-protection or cross-enhancement Phenomenon by which infection with a pathogen (or serotype) either makes a secondary infection less (protection) or more (enhancement) likely

Deterministic models Models that do not take chance events into account and approximate the occurrence of events by the average or expected rate

Disease ecology The study of disease as species that colonize niches (usually hosts, but also intermediate environmental stages or secondary hosts)

Endemic transmission Continual transmission of disease, which may still vary for reasons such as seasons or immunization, but does not depend on external introduction of the pathogen

Evolutionary stable (strategy) An ESS is a trait value or a combination of values that cannot be beaten by any other, and therefore, once achieved, it stays the same in the population (provided the environment does not change). If more than one organism is evolving in response to the others, a coevolutionary stable strategy, CSS, can be achieved, so that individuals of any species with different trait values cannot succeed in the population better than the ones that have achieved it

F_{IS}, F_{ST} Measures of genetic differentiation. F_{IS} is the inbreeding coefficient, which is a measure of consanguinity, or relationship. F_{ST} is a measure of variation within a subpopulation compared to the variation in the total population

Fitness The capacity of an individual or population to propagate and persist in the population fitness has many components such as fecundity (the more offspring a variant has, the more successful it will be), survival (the better it survives, the more it will be present), and many others

Genetic background The genetic sequence of an individual. Clones of a laboratory animal share a same genetic background

Heritable genetic variation The distribution in a population of variants of any genes, i.e., variants that can be transmitted genetically from parent to offspring

Incidence (of disease) The number of new infections in a given time period, e.g., the weekly incidence of dengue fever is the number of new cases of the disease in a particular week (also usually defined for a geographic region, like a city)

Least-squares fitting Frequentist estimation method that minimizes the square of the distance between the data points and a curve (straight line, function, or model) and should explain the trends observed

Neutral evolution Evolution that does not depend on natural selection, most readily exemplified by sequence variation that has no function and therefore does not affect survival, i.e., is neutral

Phenotypic evolution Evolution of observable traits, usually equated to evolution of traits under selection

Phenotypic variation Distribution of trait values, e.g., height in a human population

Polymorphism Variation. A polymorphic gene is a gene that differs in individuals (or the pair of chromosomes of a single individual)

Prevalence (of disease) The number of disease cases in a certain point in time

Priming (Immune system) Upregulating immune responses that would then respond more readily to an aggression, e.g., exposing the immune system to a bacterium may activate responses that would then help kill viruses introduced afterwards

Random genetic drift Process by which, due to chance in reproduction, some individuals pass on offspring (and therefore genes) to the next generation, while others do not. As a consequence, over some time, there is a finite probability that some genes get lost and others get fixed without there being any selection for them. In Motoo Kimura's words, it is the "survival of the luckiest"

Reciprocal interactions, Feedback Any mechanism that allows a process to self-regulate or self-enhance is termed feedback; ecology affects evolution, and in the case where evolution of traits affects ecology in the short term, it is said that the relationship between ecology and evolution is reciprocal

Selection coefficient The mathematical representation of selection, usually as a single parameter (s)

Selection, selective pressure Any process that favors specific variants in the population, e.g., pesticides favor the survival of resistant pests, and therefore selects them for future generations

Serotype Pathogen type that elicits a specific immune response and therefore is distinguished from similar pathogens based on its antigens and matching antibodies produced against them

Statistical inference, fitting Any method that adjusts free parameters of a curve (function or model) to find the values that best explain the observed data, i.e., that best "fits" the data

Sylvatic transmission cycle Disease transmission that happens among wild animals, independently from humans

Temporary cross-immunity Temporary cross-protection that wanes after some time

Theoretical epidemiology Broadly similar to disease ecology, but associated more with the theoretical aspects of the practice of epidemiology, as opposed to the study of diversity of pathogens

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