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The evolution of transmission mode

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Summary

This article reviews research on the evolutionary mechanisms leading to different transmission modes. Such modes are often under genetic control of the host or the pathogen, and often in conflict with each other via trade-offs. Among strain variation in transmission mode and virulence, and among host susceptibility to transmission mode are to be expected. Evolutionary changes in transmission mode have been inferred through experimental and phylogenetic studies, including changes in transmission associated with host-shifts and with evolution of the unusually complex life cycles of many parasites. Understanding the forces that determine the evolution of particular transmission modes presents a fascinating medley of problems, for which there is a lack of good data and often a lack of conceptual understanding or appropriate methodologies. Our best information comes from studies that have been focused on the vertical vs. horizontal transmission dichotomy, but we currently have few guidelines whereby we can determine with any degree of confidence whether, when and how rapidly new transmission modes may evolve. Obtaining such knowledge is a matter of urgency in relation to extant disease threats.

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1. Introduction

Transmission is central to disease biology and epidemiology, and the transmission modes of pathogens and parasites are complex and diverse. However, there has been limited attention given to how transmission mode evolves, especially in comparison with other evolutionary outcomes of disease interactions such as co-evolution during the infection process (Boots et al. 2014)[1], the evolution of host-range (Antonovics et al. 2011)[2], or the evolution of virulence (Ewald 1983[3]; Bull and Lauring 2014) [4]. This review examines major issues and findings relating to the evolution of transmission mode. We focus on the evolution of transmission as a trait in its own right, and only tangentially consider how different transmission modes once established have evolutionary consequences disease expression and virulence as these have been the subject of other reviews (Ewald 1983 [3], 1994; Lockhart et al. 1996 [5]; Moore 2002[6]; Moran et al. 2008[7]; Ebert 2013[8]) ,

Our review broadly addresses the following questions:

- What are the types of transmission and how can they be studied? We address some awkward semantic and methodological problems unique to studying transmission modes and routes.
- How does transmission mode evolve? At a micro-evolutionary scale, we examine the evidence for genetic variation in transmission mode and the nature of the trade-offs involved, including evidence from selection experiments.
- What are the predictions of population genetic models about directions of evolution in transmission mode? When will there be stable genetic variation for transmission mode and when will mixed modes be favored?
- What directions has the evolution of transmission mode made in the past? We review phylogenetic and comparative studies on changes in transmission mode, asking if there are preferred evolutionary pathways, and what forces might lead to them.
- Do changes in transmission mode accompany host-shifts or emergence of new diseases? We examine the evidence for this, and emphasize the importance of understanding this process in dealing with newly emerging diseases.
- Throughout we emphasize that the evolution of 'transmission mode' is determined by the genotype of the pathogen and the host, and is a co-evolutionary process and not just an evolved property of the pathogen.

2. Transmission modes and routes

The transmission of parasites and pathogens is often categorized in the literature and public health web sites into 'modes' and 'routes'; however, these two terms are used interchangeably which confuses two concepts which are important for thinking about the process whereby transmission evolves. By analogy with common usage, we can think of the difference between a 'mode' of transport (e.g. train, bus, car, bicycle) and a 'route' taken to get to a destination (e.g. via which city, or via which specific international departure and arrival point – with the customs serving perhaps as the immune system!). We could therefore think of 'mode' referring to the method that a pathogen uses to get from starting point to destination, whereas the 'route' is the path taken using the chosen mode and includes a starting point (site of pathogen presentation, or portal of exit), a specific pathway used, and a destination (where the pathogen enters). This distinction is important because the mode defines certain epidemiological characteristics of the pathogen and the disease, and expectations for its possible evolution; for example, sexual vs non-sexual transmission (Antonovics et al 2011). The routes for one mode may be several, or many, and tells specifically *how* the pathogen will actually get out of one body and infect another, e.g. fecal-oral, hand-oral, fomite-lung, etc. (of course knowing the route still does not tell you the mechanisms of infection which are also incredibly varied!). Until we know both the mode and route, the transmission is not fully defined; e.g. a

pathogen transmitted by the lung-to-lung route may be droplet borne or airborne; a pathogen transmitted by the vertical mode may take the transplacental or vaginal-skin route. Once we know both, the evolutionary trajectory may be hypothesized and control measures discussed. Knowing the variety of routes that a mode has might also indicate how restricted a particular pathogen might be in its transmission, and suggest precise, or more wide-ranging methods of control; e.g. airborne pathogens mainly spread from on respiratory tract to another, whereas vector-borne pathogens can be transmitted from vector to skin, from the vectors faeces to lung, or from a vector bite to the blood stream.

Modes can be subcategorized in various ways: one way is shown in Table 1. The actual hierarchical order of the divisions and sub-divisions is debatable but these are the commonly used dichotomies when describing 'transmission modes'. Within the evolutionary literature on disease, the major distinction among transmission modes is between vertical and horizontal transmission, with horizontal transmission commonly subdivided into sexual vs. non-sexual. Most health and government organisations classify infectious diseases as being transmitted 'directly' (e.g. sexual, vertical, skin-to-skin contact) and 'indirectly' (e.g. airborne, vector borne, vehicle borne, water and food borne) (CDC 2012,[9] ECDC 2016[10], WHO 2016[11]). Since directly transmittable diseases are by definition spread by human-to-human contact, this distinction may be useful to warn medical workers dealing with directly transmitted pathogens that they may be at risk of infection from patients. Another distinction is sometimes made based on the form of the transmission function in relation to density of infected individuals, namely frequency-dependent vs. density-dependent transmission (Getz and Pickering 1993)[12].

Surprisingly, the terms 'movement' and 'dispersal' appear rarely in the disease literature, and are generally considered to be processes that are component of transmission. Pocock et al. (2005)[13] define dispersal as movement of an individual from a source location to a new location, with 'effective dispersal' including the added element of establishment and breeding in the new location. Therefore, transmission in the disease literature corresponds to the idea of effective dispersal in the ecological sphere.

3. Determining transmission modes

Quantifying the contribution of different modes and routes to overall transmission of a pathogen is a major challenge, and the general lack of data on transmission for most pathogens is one of the greatest obstacles to studying its evolution. For example, as discussed below, understanding evolutionary pathways in transmission mode is more limited by reliable knowledge of the transmission mode than by the phylogenies of the pathogens involved (Antonovics et al. 2011)[14]. Generally, three approaches have been taken to establish and measure transmission mode: genetic studies involving markers, observation of contact processes, and experimental studies. The presence of congruent host and pathogen phylogenies has also been used to infer that in the past pathogen transmission has been predominantly vertical (Hafner et al. 1994[15]; Moran et al. 2008)[16]. However, this interpretation has been questioned because congruent phylogenies may also result from the greater likelihood of host shifts between related taxa by horizontal transmission (DeVienne et al. 2007[17], 2013[18]; Charleston and Robertson 2002[19]). Moreover, high vertical transmission does not preclude horizontal transmission pathways as the latter may be essential to maintain a high disease prevalence, in turn resulting in high effective vertical transmission (Lipsitch et al. 1995).[20]

Most infectious diseases have the potential to be transmitted by multiple modes, so a major issue is determining which modes are the most important in a particular host-pathogen system. Even routes that appear "incidental" or unimportant, may, if they have a genetic basis, be the target of selection in novel circumstances. A classic example is the protozoan *Toxoplasma gondii*. Whilst the one definitive host, a species of Felidae, sheds oocysts in the stool, these are highly resistant and can infect all warm blooded organisms when they consume contaminated vegetation or raw meat. Species such as sheep, humans and other animals, in particular mice and rats, can maintain infection through congenital or neonate transmission(Rejmanek, et al. 2010[21]; Webster 1994[22]), and several cases of

sexual transmission has also been documented in experimental studies (Dass, et al. 2011[23]; (Arantes, et al. 2009)[24];)s ; Wanderley, et al. 2013[25]). Another example is Rift Valley fever virus which spreads among livestock through mosquitoes bites but can also be transmitted vertically (Antonis, et al. 2013[26]). From domestic animals, RVFV is transmitted to humans mainly through direct contact with infected animals, consumption of raw milk, and in few cases, transmission through mosquito bites (LaBeaud, et al. 2008[27]; Seufi and Galal 2010[28]). During inter-epidemic periods, RVFV may be maintained by transovarial vertical transmission in some mosquito species (Favier, et al. 2006[29]). However, as with *Toxoplasma*, we know little about the strength of these different routes of transmission, and whether any of them involve unique genetic variants.

At first sight, epidemiological tracing using genetic markers might seem a particularly useful approach to studying transmission mode, but while they can identify the source and target of a transmission event they cannot *per se* pinpoint the transmission route unless combined with other approaches. A classic example is the tracing of HIV infections to particular health care workers, and establishing that such infections had to be blood borne rather than sexually transmitted based on associated risk factors such as known history of sexual activity (Ou et al. 1992[30]). A more recent example relates to the outbreaks of foot and mouth disease (FMDV) in the United Kingdom in August 2007. Genetic studies showed that these were caused by a virus strain handled at two FMD laboratories located at the Pirbright Institute for viral research in the UK. Sequencing of viral genomes revealed a chain of transmission events from inside the institute to cattle in the vicinity, but the environemntal pathways responsible were determined by careful analysis of protocols and handling of waste material (Cottam, et al. 2008)[31].

Genetic markers are perhaps most useful in determining transmission routes in multi-host systems. For example, microsatellites have been used to identify possible hosts of *Schistosoma japonicum* (Rudge, et al. 2008[32]; Rudge, et al. 2009[33]; Wang, et al. 2006[34]). PCR analysis of mosquito blood meals was used to establish which bird species were potentially important for West Nile Virus transmission to humans (Kilpatrick et al. 2006[35]). Studies of co-inheritance of genetic markers in parasites and both cytoplasmic and nuclear genetic markers in their hosts can also inform the degree to which transmission is vertical or horizontal (Wade 2007)[36]. Under complete vertical transmission there should be compete linkage disequilibrium between alleles at host and pathogen loci, and degrees of departure from this can be used to back-infer the amount of horizontal transfer (Stewart et al. 2008)[37]. Therefore, whilst genetic markers can pinpoint some general pathways of pathogen transmission and identify pathpgen sources, they cannot by themselves reliably distinguish between mode of transmission and its relative importance under differing scenarios.

Experimental infections can also inform estimates of relative importance of different transmission routes. For example, in avian influenza, experimental infections have estimated persistence of virus in the environment, and thus the relative importance of aerosol vs fecal-oral route (Bouma, et al. 2009[38]; Shortridge, et al. 1998[39]; Spekreijse, et al. 2011[40]). Similarly, experimental studies on FMDV have used groups of calves either exposed infected individuals directly or housed them in buildings that had previously held infected individuals to study the relative importance of direct vs. environmental transmission (Rueda and al 2015[41]). As another example, to determine whether the congenital transmission route alone was sufficient to maintain transmission of *Toxoplasma gondii* in brown rats, *Rattus norvegicus*, (Webster 1994[42]), rats were trapped from local farms and released into a large naturalistic outdoor enclosure in the absence of any oocysts from the feline definitive host or contaminated meat (the rodent's feed was supplemented with cereals only). Over the subsequent three years, the rat population expanded but the seroprevalence remained approximately constant, showing that feline hosts were not essential to maintain transmission. Although entomopathogenic *Rickettsia* has generally been assumed to be vertically transmitted, experimental studies, Caspi-Fluger et al.(2012[43]), showed that a phytophagous rickettsia could be horizontally transmitted via the phloem; uninfected whiteflies of *Bimesa tabaci* segregated from infected whiteflies could acquire the infection by feeding on the same leaf.

Experimental studies exposing potential arthropod vectors to pathogen by allowing them to feed on infected hosts are relatively commonplace. The detection of the pathogens (often viral RNA) can be in the saliva or head of the insect (Dubrulle et al. 2009[44]; De Regge et al. 2012[45]) or in the whole insect (Sabio et al. 2006[46]; Mehlhorn et al. 2007[47]). However, most such studies implicitly assume that the demonstration of pathogen replication in a vector following artificial exposure to a pathogen is adequate to infer vector-borne transmission in the field. Unfortunately, studying actual transmission under field conditions is both expensive and time-consuming, and so is rarely done (Anderson et al. 2010)[48].

Transmission mode can obviously be determined by many methods. Contact tracing and inferring transmission routes based on behaviors among these contacts is a method commonly used in humans. Age specificity of infection, location of the pathogen, site of the lesions, and the biology of the transmission stages are all pointers to the transmission mode. While these methods are important in identifying routes, and important in directing control measures in human and agricultural diseases, quantifying the amount of transmission by the different routes always remains a challenge for there is no simple answer.

4. Genetic variation in transmission mode

The very diverse transmission modes that occur in closely related pathogen species suggests that the evolution of new transmission routes is ongoing and likely commonplace in nature. For example, many closely related strains of sexually transmitted diseases having both sexual and non-sexual transmission (Thrall and Antonovics 1997[49]; Antonovics *et al.* 2011[14]). However, it is often not clear if transitions to a given transmission mode are simply the product of the host ecology and unrelated to genetic change. Quite drastic changes in transmission mode may not be contingent on any or only very little genetic change; the difficulty of distinguishing *Trypanosoma equiperdum* (causing dourine, a sexually transmitted disease in horses) from *T. brucei* (causing sleeping sickness transmitted by tsetse flies) suggests this host shift and transmission mode may have been possible with very little underlying genetic change (Sanchez et al. 2015)[50].

Some of the best evidence we have for a genetic basis for transmission mode is the demonstration of specific genetic pathways leading to different tissue tropisms in closely related strains or species with contrasting transmission modes, e.g. genital and ocular chlamydia (Caldwell *et al.* 2003[51], Antonovics *et al.* 2011[14]). However, given the difficulty of quantifying transmission modes, it is perhaps not surprising that there appear to have been almost no studies on the quantitative genetics of transmission mode. Evidence of genetic control of transmission mode comes from the study of fungal endophytes that often act as partial "parasitic castrators" producing fruiting bodies on the plant inflorescence (which produce horizontally transmitted spores), and whose hyphae invade the seeds, resultig in vertically transmitted through the seed. Kover and Clay 1998[52] showed that fungal strains of *Atkinsonella* differed in the degree to which they induced fruiting bodies; but their vertical transmission was not studied. Tintjer et al. 2008[53] showed that cloned genotypes of the grass *Elymus hystrix,* when infected with the fungus *Epichloë elymi* differed in the degree to which they produced fungal fruiting bodies responsible for horizontal transmission; however, all genotypes showed close to 100% vertical transmission of the fungus to the seeds, so there was no evidence of a trade-off with vertical transmission. These studies clearly show the importance of host factors in determining transmission mode.

Experimental studies have manipulated levels of horizontal and vertical transmission, and studied associated changes in the pathogen. Stewart et al. 2005[54] passaged barley stripe mosaic virus (BSMV) in its host, barley (*Hordeum vulgare*) horizontally for four host generations and vertically for three generations. Each selection regime resulted in an increase in transmissibility by the respective route, with clear trade-offs between them. In keeping with theoretical expectations, an increase in virulence by the horizontal route and a decrease in virulence by the vertical route, although levels of viral virulence did not reflect viral titer in the plants. Bull et al. (1991[55]) manipulated opportunities for vertical or horizontal transmission of bacteriophages infecting bacteria

and found that when vertical transmission was promoted the viruses became less virulent. Similarly, Pagan et al. (2014[56]) selected for reduced pathogen virulence by serially passaging cucumber mosaic virus vertically in its host *Arabidopsis thaliana*, but no selection response was observed following horizontal transmission. Using the bacterium *Holospora undulata* infecting the protozoan *Paramecium caudatum*, Magalon et al. (2010[57]) demonstrated that populations of the host maintained below their carrying capacity selected for increased vertical transmission of the bacterium since high birth rates increased opportunities for vertical transmission. Dusi et al. (2015[58]) then showed that the bacteria evolved in conditions promoting vertical transmission exhibited an almost complete loss of infectivity via the horizontal transmission route. Phage λ viruses have a "genetic switch" which, in one state, keeps them as a prophage in the *E. coli* genome so they are vertically transmitted, but in the alternate state gets turned on in response to stress, which initiates cell lysis and horizontal transmission. Refardt and Rainey 2010[59] showed that the sensitivity and threshold of this switch responds quickly to selection. However, the outcome of selection experiments is also not always as expected; Turner et al. (1989[60]) allowed plasmids to evolve for 500 generations in populations of bacteria that differed in density, but there was no evidence of response to selection for vertical or horizontal transmission.

5. Trade-offs and transmission modes

While it would be obviously advantageous for a pathogen to use all possible transmission routes, as in any evolutionary process involving a complex phenotype, there are likely to be direct trade-offs between these routes or these routes may themselves have other fitness effects. In an evolutionary context, trade-offs are quantified by measuring the genetic correlations between different traits: a negative genetic correlation between alternative transmission modes suggests increasing one transmission mode would decrease the other. However, we know of no data on estimates of genetic correlations between transmission mode and other fitness components, in either pathogens or hosts.

It has been commonplace in theoretical and general discussion to expect trade-offs in transmission mode. This is most obvious in the conflict between vertical and horizontal transmission. Activities of a host or parasite that increase the rate of horizontal transmission (e.g., greater production of infectious particles) may increase mortality or decrease reproduction, and this will correspondingly reduce vertical transmission of the parasite via the offspring, necessarily leading to an evolutionary trade-off (Levin and Lenski 1983[61]; May and Anderson 1983[62]; Bull et al. 1991[63]; Ewald 1994[64]). Correspondingly, theory predicts that there should be a trade-off between pathogen virulence and transmission mode (Alizon et al. 2009[65]). If the pathogen kills the host quickly there is a cost in terms of a reduced number of infectious particles, which decreases horizontal transmission. At low host densities contact rates between host and pathogen may drop below the threshold necessary for persistence (Anderson and May 1981[66]), so that persistence is more likely if the pathogen can be vertically transmitted and has a low virulence so the host survives till reproduction.

These concepts seem intuitive when considering, for example, the insect baculoviruses, which exhibit both modes of transmission and are invariably lethal when horizontally transmitted, but are largely asymptomatic when vertically transmitted (Burden et al. 2002[67]). Natural populations of insects are often characterised by large seasonal variation in abundance, including a complete absence of stages that transmit horizontally; hence, such populations harbour covert baculovirus infections that are vertically transmitted (Burden at al. 2003[68]). Another example is the protozoan parasite *Ophryocystis elektroscirrha* of monarch butterflies which is transmitted horizontally when adult butterflies ingest spores on host plant leaves, and vertically when spores are transmitted on the outside of the eggs (de Roode *et al.* 2008[69]). Strains of the parasite that produce large numbers of spores cause severe infections of the larvae and pupae that reduce vertical transmission because few larvae successfully pupate. However, these strains are efficiently horizontally transmitted because they leave more spores on the leaves. Similar trade-offs are seen in a wide range of host-pathogen

systems, from malaria (Dearsly et al 1990[70]), to microsporidia (Ebert and Mangin 1997[71]) and bacteriophage (Bull *et al.* 1991[72]).

The shape of the trade-off is likely to be important in determining whether evolutionary changes lead to predominantly one mixed mode, or maintain both modes as genetic variants with alternative pathways (Ferdy and Godelle, 2005) [73]; as they (p. 623) have stated "Must we care about the …shape of the resulting trade-off…? The answer is (unfortunately) yes." The 'unfortunately' is not only because the trade-off shape is critical in determining the outcome of evolutionary predicitons, but also because the measurement of the shape of the trade-off presents particular challenges; estimates of genetic correlations *per se* cannot incorporate non-linearities (other than by transformation), and so we do not have the statistical tools for estimating non linear genetic trade-offs. The shape of the trade-off curve is also critical in determining the outcomes co-evolution of hosts and pathogens with regard to resistance and infectivity (Boots and Haraguchi 1999[74]; Baker and Antonovics 2012[75]).

The dependency of trade-offs on environmental conditions also needs to be considered. Intriguingly, research on microsporidians in mosquitoes has shown that the factors influencing selection on vertical versus horizontal transmission include food availability and whether the parasites are embedded in coinfections (Duncan et al. 2015[76]). Long-term environmental changes in SO_2 levels, by affecting the likelihood of infection via leaves, has been posited as the cause of shifts between leaf-to-leaf (horizontal) and seed (vertical) transmission of the fungal pathogen of wheat, *Phaeosphaeria nodorum* (van der Bosch *et al.* 2010[77]).

6. Evolutionary pathways in transmission mode

(a) Population Genetics Theory

While there have been many studies positing the advantages or otherwise of different transmission modes, few studies have addressed the evolution of transmission mode from a population genetics standpoint, asking how allele frequencies determining transmission mode are likely to change, and with what outcome. Thrall and Antonovics (1997)[78], observing that sexually transmitted diseases (such as chlamydia, syphilis, HSV-2, and pubic lice) often have non-sexually transmitted counterparts (strains or closely related species), asked whether it was possible to maintain genetic polymorphisms in transmission mode even when the strains excluded each other (directly or immunologically) from a single host. They implicitly assumed a complete trade-off in transmission mode, such that each genotypes could transmit either sexually (in a frequency dependent manner) or non-sexually (in a density dependent manner), and showed that stable genetic polymorphism in alternative transmission modes was possible. This was even when the pathogen strains were excluding each other on the same host resource, illustrating how "Gause's Principle" (that two species using the same resource cannot coexist) could be violated by the complexities of transmission.

There have also been applications of adaptive dynamics theory to transmission mode evolution. In a thorough analysis of the evolution of vertical vs. horizontal transmission, Ferdy and Godelle (2005)[79] examined the consequences of different forms of the trade-off between vertical and horizontal transmission. They too showed that polymorphism in transmission mode was possible if the trade-off was convex (e.g., increased horizontal transmission, if it causes sterility, will not continue to decrease vertical transmission proportionately); but if the trade-off was concave, then mixed-mode transmission of one genotype is favoured (e.g. in a situation where increased horizontal transmission that increases mortality continues to decrease vertical transmission). Their model included competition among the symbionts for resources within the host, and this complicates the outcomes, depending on the nature of the vertical vs. horizontal interaction within the host.

The evolution of transmission mode in context of virulence is important from an applied perspective. Thus if highly virulent strains can co-exist with non-virulent ones, very serious health consequences of disease in a subset of the population may be due to virulent pathogen variants. This may be less desirable than the presence of only one strain of intermediate virulence. Boldin and Kisdi

(2012)[80] investigated this in diseases that had both environmental and direct host-to-host transmission, the worry being that environmentally transmitted genotypes might show higher virulence, as their persistence would be less compromised by a shortened host life-span. Here too, stable genetic polymorphisms could be maintained; however, the polymorphism generally involved strains less virulent than would be expected under one transmission mode or the other. Van den Bosch et al (2010)[81] used a similar approach to investigate a system where there could be different level of vertical (seed) vs. horizontal (leaf-to-leaf) transmission; their model was then applied to a fungal disease (*Phaeosphaeria*) of wheat. They showed evolutionary "bi-stability" in pathogen "aggressiveness" (i.e. disease severity or virulence), and therefore the potential for polymorphisms in degree of vertical (seed) and horizontal (leaf) transmission mode under a wide range of conditions.

Several things stand out from these theoretical studies. The first is that the evolution of transmission mode has received very little attention from population geneticists, even though the results can often be illuminating theoretically and of applied significance in understanding strain variation in virulence. Polymorphisms in transmission mode and therefore virulence are possible, but the circumstances under which polymorphic genotypes vs. multiple transmission modes in one genotype are favoured remain undefined; this stands in strong contrast with our understanding of the evolution of host-pathogen interactions in infectivity and resistance [1]. Additionally, it should be noted that all the studies have assumed that transmission is under "pathogen control", i.e. that it is genetic variation in the pathogen rather than in the host that is driving the evolution of transmission mode. It remains to be seen whether more complex "transmission-genetics" makes possible co-evolutionary scenarios, in a way analogous to what is seen with genetics of resistance and infectivity.

(b) Examples of evolutionary changes in transmission mode

The general perceived 'adaptationist' wisdom is that transmission mode will evolve in the direction of where there is the greatest transmission opportunity at least cost (i.e. the mode and route that produces the greatest fitness gains for the pathogen). For example, it has been argued that decreasing density, or periods of low density will favour vertical (Ebert 2013)[82] or sexual-transmission (Smith and Dobson 1992)[83], while high density will favour aerial or (non-sexual) direct contact transmission. However, there will also be selection on hosts to decrease transmission, and the force of this selection will differ among transmission modes. For example, in primates, several immunological parameters appear to be determined largely by the degree of sexual transmission rather than by other transmission modes (Nunn et al. 2000)[84]. Moreover, if there are two potential pathways, such as ocular or genital transmission, it may be easier/less costly for the host to evolve resistance via one route rather than another. Age specificity of resistance may also determine whether a disease is transmitted aerially to offspring or sexually via reproduction among adults (Bruns et al. 2016)[85].

In the next sections we review studies that address how evolutionary changes in transmission mode may have occurred in the past. Most of them have focused on pathogens as the anticipated driver of transmission mode.

Vertical vs. horizontal transmission

Sachs et al. (2011)[86] reviewed the evolutionary transitions within bacterial symbionts, focusing mostly on mutualistic relationships. They concluded that free-living forms preceded host associated ones and that "the most basal form of transmission is horizontal transmission and likely occurs when bacteria are acquired from environmental pools." Exclusive vertical transmission was rare (of 129 host associated bacteria, 100 were horizontally transmitted, 14 vertically transmitted, and 5 had mixed mode transmission). Of the vertically transmitted species, 3 were considered to be parasitic, 11 mutualistic. Sachs suggested "vertical transmission is often an irreversible evolutionary endpoint." However, in *Rickettsia* (Perlman *et al.* 2006)[87] showed that while most species are vertically transmitted symbionts of invertebrates, some have later become horizontally (by invertebrate vectors) transmitted pathogens of vertebrates. The comparison between *Coxiella burnetii* and *Coxiella*-like endosymbionts of ticks is also relevant. *Coxiella*-like bacteria are maternally inherited, potentially mutualistic bacteria in ticks. *Coxiella burnetii* causes Quary fever (Q fever) in

humans and infects a variety of vertebrate species and is transmitted horizontally through many different routes. Recent studies have shown that *C. burnetii* recently evolved from an inherited symbiont of ticks which succeeded in infecting vertebrate cells, likely by the acquisition of novel virulence factors (Duron *et al*. 2015)[88].

Sexual vs. nonsexual transmission

From general considerations, there are arguments for expecting sexual transmission to be ancestral: frequency-dependent transmission allows the persistence of pathogens at low population densities, and therefore protects against bottleneck events; sexually transmitted diseases are often persistent in the host, and this increases their likelihood of being carried with a host migrating to a new location; and because sexual reproduction is a regular feature of the life cycle, sexual transmission may be considered relatively "reliable." On the other hand, being sexually transmitted severely limits opportunities for cross-species transmission (sexually transmitted pathogens have lower host ranges, Lockhart et al. 1996[89]), which might be a critical factor in determining long-term persistence on alternative hosts. Antonovics et al. (2011)[90] explored whether sexual transmission was ancestral or derived by mapping transmission mode onto phylogenies of pathogens. The results showed that it seemed more common for sexual transmission to be a derived trait rather than ancestral, and also that sexual transmission appeared to have evolved in an extremely diverse way, and often repeatedly as in the *Chlamydias* and HPVs. However, determination of the evolutionary pathways was very difficult because of a lack of reliable phylogenies and, as a significantly greater obstacle, a lack of accurate/reliable information on transmission mode.

Evolution of complex life-cycles in helminths

Complex life-cycles, where several life stages of a parasite are found in different hosts, is a remarkable feature of both animals and plant parasites. The hosts in such life-cycles can be extremely unrelated phylogenetically, making it hard to envisage how such 'host-shifts' could ever occur. Moreover, the occurrence of a parasite on phylogenetically distinct hosts raises the question of whether the more ancestral host represents the "original" host; alternatively, it can be posited that the original host is the "definitive" host (i.e. in which sexual reproduction occurs) and that the non-definitive host has been acquired subsequently for the asexual stages. For example, did helminths, which alternate between sexual stages in the vertebrate host and asexual stages in snails, evolve parasitism in vertebrates and then acquire the snail hosts, or were they originally parasites of molluscs?

Many authors have speculated on the pathways whereby parasites could gain new hosts and establishing complex life cycles. Much of the focus has been on the helminths (flaworms, tapeworms and nematodes) where this pattern is very prevalent (Choisy et al. 2003[91]; Parker et al. 2003[92]; Poulin 2007)[93]. For instance, parasites of the original host species may evolve to exploit that species predators, a process that has been termed "upward incorporation," and might be driven by increased parasite fecundity in larger predator hosts. For example, upward incorporation appears to have occurred when an acanthocephalan ancestor, endoparasites of marine arthropods, incorporated a vertebrate predator as a second host (Near et al. 1998[94], Herlyn et al. 2003[95]). Upward incorporation to a new definitive hosts may also increase parasite densities, and lead to an increased probability of finding a sexual partner (Brown et al. 2001[96]; Parker et al. 2015[97]) or to decrease in inbreeding because of multiple infections of a larger host (Rauch et al. 2005)[98]. In helminths, acquisition of a second intermediate (paratenic) host may also enable an increased intermixture of genotypes from the snail host within which the parasites multiply only asexually. The difficulty of accounting for such life-cycles has also led to some extreme hypotheses. For example, (Smith Trail 1980)[99] proposed that infected hosts might benefit by "submitting to" predation if suicide is repaid by inclusive fitness gains when close relatives experience reduced infection. Subsequently, parasite survival in the host's predator generated a complex life cycle by upward incorporation.

Alternatively, when the new hosts is at a lower trophic level, there may have been "downward incorporation" (Parker et al. 2003)[100]. Prey of the original host and living with it, may frequently have ingested parasite transmission stages and thereby may have become intermediate hosts. Being

prey to the original host may enhance transmission back to that host (Parker et al. 2015 [89]). Such downward incorporation has been associated with the occurrence of a "trophic vacuum," i.e. difficulty of transmission of small free-living infective stages among a host at a high trophic levels where animals are large and at low density (Benesh et al. 2014)[101]. Platyhelminthes appear to present such an example of downward incorporation: the lineage ancestral to digeneans and cestodes has become parasitic in invertebrates (Park et al. 2007)[rep]. Paratenic hosts may also be acquired by downward incorporation as a means of increasing transmission (Parker et al. 2015) [89].

Intermediate hosts could also be added via "lateral incorporation" if the parasite has multiple hosts involved; in a generalist pathogen each of two parasite stages come to specialize on one of the hosts (Parker et al. 2015) [rep].

The evolution of transmission by arthropod vectors

Haematophagous arthropods such as mosquitoes and ticks transmit a broad range of microorganisms that cause disease in vertebrates. Some vector-borne pathogens can also be transmitted via other modes such as direct contact, vertical transmission, or aerosol transmission, in many cases at a low rate(for example, the dengue virus[102]). How might such a system evolve? Possible precursors to vector-borne transmission could be an exclusively arthropod pathogen which infects a dead-end vertebrate hosts and acquires the ability to cause transmissible infections; this would be equivalent to what is termed "downward" incorporation in the context of helminths. Alternatively, an exclusively vertebrate pathogen that is repeatedly ingested by an arthropod proto-vector during blood-feeding could acquires the ability to infect it; again there is a parallel here with "upward incorporation." An intermediate step here could be mechanical transmission, in which a pathogen is be transmitted by a haematophagous insect without any fitness cost as no replication occurs in the insect. Mechanical transmission is seen in a broad range of current pathogens, both vector-borne (RVFV) and non-vector-borne (anthrax). Some vector-borne pathogens have also lost the ability to be biologically transmitted altogether (*T. evansi*). The third possibility is that a pathogen may already be infecting both vertebrate and invertebrate hosts, and is initially transmitted within and between them via alternative transmission routes, but these may be restricted to only cross-species transmissions.

Phylogenetic analyses of arthropod-borne viruses (arboviruses) provide several examples of viral groups where it appears that the ancestral virus initially infected arthropods (insects, in the case of flaviviruses[103]; ticks, in the case of orbiviruses[104]), but later acquired vertebrate hosts. Subsequently these have become transmissible by yet other haematophagous arthropod groups. Reversals of this process can also occur, a study of the host associations of rhabdoviruses vectored by arthropods, showed that arthropod specific viruses had arisen, albeit rarely (Longdon et al. 2015)[105]. The evolutionary origin of another main group of arboviruses, the alphaviruses, remain unknown as thy are all known or suspected to be arthropod-borne[106]

The flaviviruses and orbiviruses most strongly to support the scenarios of the insect host being ancestral, although in the case of insectivorous vertebrate hosts it could also plausibly be explained by the third scenario since vertebrates may be orally infected by ingesting infected arthropods (JEV[107], WNV[108]). A similar evolutionary history has been reported for *Coxiella burnetii*, the causative agent of Q-fever[109].

7. Host shifts and changes in transmission mode

A large number of emerging infectious diseases are the result of parasite shifts from one host species to another (Woolhouse et al. 2005[110]; Longdon et al. 2014[111])[112]. Different modes of transmission may occur in novel host species due to host genetic, social and ecological factors affecting the epidemiological spread of the pathogen.

Understanding how transmission evolves following host shifts is of major importance when considering the emergence of infectious disease in humans. For example, Influenza A viruses jump into humans from aquatic birds where they appear to be largely spread indirectly via the fecal-oral transmission route (Kim et al. 2009)[113]. However, in mammals, influenza viruses must evolve direct

respiratory droplet transmission to successfully spread between individuals (Russell et al. 2012)[114]. These shifts in transmission are due to differences in host receptor binding, with avian influenza having to adapt to different sialic acid receptors with different tissue distributions, in mammalian hosts (Shinya et al. 2006)[115] .

HIV-1, which is largely responsible for the AIDS pandemic in humans, is the result of host shifts of viruses from chimpanzees and gorillas into humans (Sharp 2010)[116]. How SIVs (simian immunodeficiency viruses, the non-human primate forms of HIV) are transmitted in natural populations of primates is poorly understood. A study examining SIV transmission in semi-natural mandril populations found that transmission is correlated with maternal kinship yet is not transmitted marernally, suggesting behavioural interactions between related juveniles facilitate transmission (Fouchet et al. 2012)[117]. This differs from HIV in humans, where transmission is largely sexual and maternal, or through infected blood. It is not known how important SIV transmission by wounding is in primates. Surprisingly, even though HIV phylogeny is well understood, functional studies have not examined whether the change in transmission mode is due to evolutionary changes in the pathogen, or if there are simply different transmission opportunities in different host species.

Likewise, endophytic fungi from the genus *Epichloë* show evidence of divergence in transmission mode following host shifts. Different lineages of the fungi appear to have emerged through host shifts between grass species, with associated changes in reproduction and transmission mode. Some species reproduce sexually and are horizontally transmitted and others reproduce asexually and are vertically transmitted (Brem and Leuchtmann 2003)[118].

The maternally transmitted endosymbiont *Wolbachia,* uses various forms of reproductive manipulation to maximise its transmission and ensure its persistence in host populations (Engelstadter and Hurst 2009)[119]. However, as has been shown experimentally, *Wolbachia* can change phenotype directly following a host shift. For example, a *Wolbachia* strain that causes cytoplasmic incompatibility in *Drosophila recens* causes males to die in a new host, *D. subquinaria* (Jaenike 2007)[120]. A similar change has been observed in a host shift of *Wolbachia* between two species of Lepidoptera (Sasaki et al. 2002)[121], and the inverse pattern in shifts of male killing strains when they are moved into different *Drosophila* species (Veneti et al. 2012)[122]. These changes in phenotype seem to be due to host factors and the expression of existing genotypes rather than *de novo* evolution of the pathogens/symbionts, and suggests that they maintain the genetic capability to express multiple modes of transmission. A study of five virus families found that viral speciation events were primarily associated with host shifts rather than with changes in tissue tropism within the host (Kitchen et al. 2011)[123]. Similar tissue tropisms suggest similar routes of transmission rather than changes in transmission mode by the pathogen.

8. Evolution of transmission mode and human disease

Changes in transmission mode are often involved in disease emergence, and it remains a matter of urgency to determine with confidence whether new transmission modes may evolve in extant disease threats or if minority transmission modes could become major routes given new circumstances and opportunities. Thus in the recent Ebola epidemic there were fears in the mass media that the ebola virus might evolve aerial transmission given greater opportunities for this route of transmission in crowded human situations (Ross 2014)[124], especially as aerosol transmission of filoviruses has been shown in lab experiments (Johnson et al. 1995[125], Twenhafel et al. 2013)[126]. Similarly, the possibility of sexual routes of infection of not only Ebola but also Zika virus (Mansuy et al. 2016)[127] beg the serious question of whether such routes might become more important because of evolutionary changes under new transmission opportunities. Explicit consideration of "why" particular routes of transmission do or do not evolve has been rare. Day et al. 2008[128] discussed why HIV appeared not to have evolved vector transmission (via blood meals) and, among other possibilities, argued that this was because such transmission might have been quickly lethal and therefore the pathogen would have had a low fitness. Unfortunately, we simply do not have enough knowledge of the kinds of mutational steps that would be needed for changes in transmission mode to

happen, whether such changes would have associated costs, nor of the circumstances that would favour their spread. There is clearly some urgency in addressing such issues in a rigorous way at a functional, comparative, and experimental level.

Transmission mode has strong evolutionary consequences for disease severity, and conversely changes in disease severity due to treatment could result in evolutionary changes in transmission pathways, in an analogous way to which there is concern that vaccination policies may change pathogen replication rate and virulence. There is evidence that historical changes towards reduced virulence of syphilis were associated with a shift from non-sexual to sexual transmission (Knell 2004)[129]. However, we do not know of any research that has considered this possibility. One of the largest gaps in our knowledge remains the mechanisms of evolution of transmission itself.

9. Conclusions

The evolution of transmission mode presents a fascinating medley of challenges for the future, ranging from theoretical exploration of transmission in a co-evolutionary setting, to explaining startling biological conundrums such as the evolution of complex life-cycles. It is very clear that there are many different ideas and approaches, but it is a difficult field where even simply quantifying the phenotype, i.e. the contributions of different transmission modes and routes to pathogen and host fitness, is a huge hurdle. In the context of human diseases, there is a remarkable lack of understanding "why" and "when" different transmission modes are likely to evolve, and whether changed circumstances following pathogen entry into a human population would result in the evolutionary amplification of a particular transmission pathway. This applied imperative is sufficient reason to see research into the evolution of transmission as an important continuing endeavour.

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Ethics

This work involved no human or other animal subjects.

Data Accessibility

The datasets supporting this article have been uploaded as part of the Supplementary Material.

Authors' Contributions

All authors contributed ideas and substantive written sections to the review. These were co-ordinated and edited by Antonovics. Wilson made notes on the group discussions that were the basis for the review.

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See endnotes (to be revised on submission)

Tables

Table 1. One of many possible classifications of transmission modes, to illustrate the use of the terms 'mode' and 'route', with the former term being used for the method of getting point to destination, and the latter for the path taken, which includes the point of exit and entry. The table is not intended to be definitive or comprehensive; thus, for example, vector transmission could be further sub-divided into passive, or biological, and the latter into multiplicative or non-multiplicative/circulatory-only.

MODE				ROUTE (examples)
Vertical				Cytoplasmic, transplacental, during vaginal birth or breast feeding
Horizontal	Sexual			Mainly genital-genital, but also oro-genital, flower to flower
	Non-sexual	Direct contact		skin-to-skin: kissing, biting, touching
		Airborne		respiratory tract-respiratory tract
		Indirect	Environmental	contaminated food-oral, infected water-oral, soil-oral
			Fomites	clothing-skin, needle-blood, doorknob-hand
			Vector borne	cutaneous penetration; vector fecal deposition, vector identity

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