

Research



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Linking sex differences to the evolution of infectious disease life-histories

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Sex differences in the prevalence, course and severity of infection are widespread, yet the evolutionary consequences of these differences remain unclear. Understanding how male–female differences affect the trajectory of infectious disease requires connecting the contrasting dynamics that pathogens might experience within each sex to the number of susceptible and infected individuals that are circulating in a population. In this study, we build on theory using genetic covariance functions to link the growth of a pathogen within a host to the evolution and spread of disease between individuals. Using the *Daphnia*–*Pasteuria* system as a test case, we show that on the basis of within-host dynamics alone, females seem to be more evolutionarily liable for the pathogen, with higher spore loads and greater divergence among pathogen genotypes as infection progresses. Between-host transmission, however, appears to offset the lower performance of a pathogen within a male host, making even subtle differences between the sexes evolutionarily relevant, as long as the selection generated by the between-host dynamics is sufficiently strong. Our model suggests that relatively simple differences in within-host processes occurring in males and females can lead to complex patterns of genetic constraint on pathogen evolution, particularly during an expanding epidemic.

This article is part of the theme issue 'Linking local adaptation with the evolution of sex differences'.

1. Introduction

Pathogens face many sources of variability in the hosts they can infect and will encounter individuals that differ in age, nutritional condition, body size, immune status and physiology, among other factors. Often the most contrasting conditions a pathogen will encounter arise from intrinsic differences in the environments they experience when infecting a male versus a female of a given host species. Owing to unique ways that each sex maximizes individual fitness, males and females frequently diverge in their morphology, life-history, behaviour, reproductive strategies, immune investment and genetic architecture [1–4]. Not surprisingly, these differences influence how males and females interact with pathogens. In many species, males are commonly more susceptible to infection and develop higher parasite loads than females [5], although the 'sicker sex' can vary across species and broader taxonomic groups [6–10]. The consequences of these differences for the evolution of infectious disease should be profound, as an invading pathogen needs to adapt to two very different contexts—males and females—placing fundamental limits on its own performance (i.e. [11,12]); yet the influence of male–female differences on pathogen evolution remains poorly described (for a discussion on this topic, see [13]).

Pathogen fitness, in general, is determined by processes that occur both within and between susceptible hosts. Within the host is where pathogens must proliferate, interact with host defence mechanisms and exploit host resources (for overviews, see [14,15]). Competition and conflict between pathogen strains add to this dynamic process to influence the duration of

infectiousness and the extent to which the pathogen is transmissible (reviewed in [16]). Pathogens, however, must also spread between hosts to survive. At the population level, how a pathogen shapes the numbers of susceptible, infected and resistant hosts determines the rate at which a disease spreads and the ability of a pathogen to persist in the host population over time [17,18]. Overall, pathogen evolution depends on finding a balance between these two contrasting levels of selection; a pathogen that is most successful at proliferating within a host is not necessarily the one that will generate the most new infections in the host population [19–21].

The potential for host sex to modify the evolution and epidemiology of infectious disease will thus occur at two different scales. Upon infecting either a male or female, a pathogen will experience substantial differences in many physiological parameters, from immune capacity and metabolic rate, to energy stores or body size; leading to one sex inevitably being more exploitable than the other [5,13,22,23]. The consequences that this has for the evolution of infectious disease, however, depends on connecting the dynamics that a pathogen might experience within a male or female to the number of susceptible and infected individuals that are circulating in a population [23,24]. This influence of host sex on both within- and between-host processes has yet to be empirically tested. Studies have either considered the performance of pathogens at different scales but in one sex alone or have estimated pathogen fitness in both male and female hosts, but without reference to the resulting between-host dynamics (e.g. [25] cf. [26]). It remains unknown whether increased growth and performance of a pathogen in the ‘sicker sex’ directly translates to an enhanced ability of a pathogen to spread between hosts, and ultimately a change in the evolutionary trajectory of a pathogen.

One starting point for resolving if and how the sex of a host might modify the evolution of infectious disease is to consider the evolutionary trajectory of a pathogen that exclusively encounters only male or female hosts. Detailed nested models that bridge the within- and between-host divide (e.g. [27–29]; reviewed in [19]) are difficult to apply in this context owing to a lack of information about the mechanistic basis of sex differences. Day *et al.* [30] and Mideo *et al.* [25], however, developed a function-valued trait approach (i.e. [31]) that models the evolution of infectious disease life-histories—patterns of transmission and virulence over the course of an infection—using observable estimates of pathogen performance. By tracking performance as a function of infection age, this approach estimates the evolutionary constraints that arise from either a lack of genetic variation at any age, or from genetic trade-offs between early and late ages that prevent a pathogen from maximizing performance at all times. In practice, patterns of transmission and virulence over the course of infection can be estimated for multiple pathogen genotypes and reduced to a genetic variance–covariance function that captures these underlying constraints. Rates of change in each disease trait can then be predicted by combining the genetic covariance functions with estimates of selection as generated by the epidemiological dynamics arising from transitions between susceptible and infected hosts at the population level [25,30].

In this study, we apply this genetic covariance approach to the question of how males and females might differentially influence the evolutionary trajectory of a pathogen. Our test case for linking sex differences to disease life-histories is the water flea

Daphnia magna and its bacterial pathogen, *Pasteuria ramosa* [32]. In females, infection results in a severe loss of fecundity, a reduction in lifespan and gigantism [33–35]. In contrast, males are naturally smaller, more resistant to infection, allow for fewer spores to be produced and suffer less damage from the pathogen in terms of the reduction in lifespan relative to uninfected males [26,36,37]. The duration of infection is also much shorter in males in part because of their shorter natural lifespans [26,37]. Since the host has to die to transmit the infection, this sex difference in life-history may offset the within-host growth disadvantage the pathogen has in males once between-host dynamics are explored. In *Daphnia*, much like all other species with two separate sexes, pathogen growth and performance in the ‘sicker sex’ [5] may not necessarily translate to a better spread of infection between hosts.

Our goal was to formalize these sex differences in the *Daphnia*–*Pasteuria* system. We first evaluated the dynamics of the within-host process in males and females using six genotypes of the pathogen *P. ramosa*. Infected hosts were destructively sampled, and the spore loads of the pathogen quantified at short time intervals over a period of up to 48 days. We then developed an epidemiological model that captured the key characteristics of the *Daphnia*–*Pasteuria* infection cycle and predicted the evolutionary trajectory and rate of change of within-host patterns of spore loads under the conditions of exclusively male-limited or female-limited infections. With this approach, we explored how and why epidemiological dynamics and sex-specific patterns of genetic covariance might interact to generate contrasting patterns of disease life-history evolution, providing a proof-of-concept that male–female differences are an important source of heterogeneity shaping pathogen evolution.

2. Methods

Daphnia magna Straus is a facultative parthenogenetic crustacean found in ponds and lakes throughout Eurasia and North America. Under favourable conditions, female *Daphnia* reproduce asexually, but when environmental conditions change, genetically identical male *Daphnia* and sexual resting eggs (ephippia) can be produced [38,39]. As a result, *Daphnia* populations will be predominantly female biased, but males can still constitute a large portion of the population at times during a season [40]. *Pasteuria ramosa* Metchnikoff 1888 is an endospore-forming bacteria that is horizontally transmitted, with spores released from the decaying cadaver of infected animals. For this study, we used a single host genotype (HU-HO-2) and six pathogen genotypes (C1, C14, C18, C19, C20 and C24) that differ strongly in the resulting symptoms of infectious disease [33,34,41].

Before the experiment, the *Daphnia* clones were maintained under standardized conditions for at least three generations. Animals were raised individually in 60-ml jars filled with 50 ml of artificial media (ADaM; [42] modified as per [43]) and kept in a single controlled climate chamber (16:8 light:dark cycle and 20°C). Animals were fed daily with green algae (*Scenedesmus* sp., 5 million cells per day as an adult) and transferred into fresh media twice weekly. Subsequent experimental animals were maintained under identical conditions.

(a) Generation and analysis of experimental infection data

A cross-infection experiment was conducted using the two sexes and six different *P. ramosa* genotypes as part of a full factorial

design. To produce genetically identical male and female *Daphnia* we followed the methods of Thompson *et al.* [36] and exposed mothers to a short pulse of $300 \mu\text{g l}^{-1}$ of the hormone methyl farnesoate (product ID: S-0153, Echelon Biosciences, Salt Lake City, UT, USA). The following clutches were collected and the sex of all offspring determined. This method has been shown to have no detectable impact on multiple components of host and pathogen fitness (see [36]). From these standardized cultures, animals were collected at birth and placed individually in 60-ml jars filled with 20 ml of *Daphnia* medium.

On days 4 and 5, each experimental male and female received 20 000 spores of a randomly allocated pathogen genotype (i.e. 40 000 spores in total per individual) and 60 animals received the equivalent volume of a control (placebo suspension produced from uninfected *Daphnia*). This dosing procedure ensures a high infection rate (but still less than 100%) and avoids the impact of moulting in preventing infection. On day 6, hosts were transferred to fresh 60-ml jars filled with 50 ml of ADaM and maintained under standardized conditions as outlined above. This day is counted as the first day post-exposure (DPE). For each pathogen genotype, we sampled up to six individuals every 2 days, plus any that had died recently. This began at 18 DPE, as counting of fully developed spores is unreliable before this time. In total, there were 12 treatment groups (two sexes $\times$ 6 pathogen genotypes), with up to 250 animals initially assigned to each pathogen genotype, plus 60 unexposed controls per host sex. During the experiment, none of the control individuals became infected and will not be considered further.

From this collection, we recorded the spore loads for up to 20 randomly sampled animals per age (range 2 to 20, total 1578 samples). The last possible sampling day before the supply of infected animal was exhausted ranged between 26 to 38 DPE for males and 32 to 48 DPE for females. Spore loads were measured using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA), with custom gates based on fluorescence (FL3) and side scatter (SSA) used to isolate mature transmission ready spores from algae, animal debris or immature spores (see also [26]). Finally, a two-factor analysis of covariance was used to test whether the increase in spore loads with age since exposure differed between males and females, the pathogen genotypes or with an interaction between these two factors. Before analysis, pathogen spore loads were square-root transformed for hypothesis testing, although the regression parameters and figures are presented on the original scale for ease of interpretation. All statistical analyses were performed in R (v. 3.4.3; available at: www.r-project.org).

(b) Definitions of transmission and virulence

Transmission of *Pasteuria* occurs through the production of mature, dormant spores that are released into the environment from decaying cadavers of infected hosts [32]. Spores of this pathogen persist in the sediment or water column of the environment and are taken up passively by their *Daphnia* host via filter feeding, entering the host via attachment to the oesophagus. Empirical work has suggested that the likelihood of infection conforms to the mass-action principle: a higher density of mature spores encountered leads to a greater chance of becoming infected [44,45]. Once an infection has established, the pathogen proliferates via a series of developmental stages that culminate in the production of the mature spores and the eventual death of the host [32,34]. However, the first mature spores do not appear until 15–18 days post initial infection, after which spore density increases continuously until the pathogen terminates the infection 30 days later or more under laboratory conditions [34]. Expected time to death following infection is shorter in males than females, in part owing to the lower average lifespan

of healthy males (33 days for males versus 67 days for females [36]).

In the following models, we explore how transmission will vary between the sexes owing to combined differences in both the rate at which mature spores are produced and the time taken until host death following infection. We assume that the rate of pathogen transmission from hosts that died at infection age a is proportional to the within-host pathogen spore density at that infection age (see electronic supplementary material, table S1), and that virulence is the rate of disease-induced mortality in the host, which is constant over the duration of the infection.

We consider two scenarios that describe differences in the *per capita* rate at which infections end in each sex. (i) Simplified mortality, where rates vary only with the sex of the host, based on previous findings that the expected time to death following infection is shorter in males than females [26,36,37]. Here we assume that mortality rates in males are 50% higher than in females (see electronic supplementary material, table S3), resulting in most infected males (99%) dying roughly 8 days sooner than most infected females. We apply this constant mortality rate to the full duration of observed infection in any pathogen genotype–host sex combination, thereby extrapolating spore loads to ages beyond the observed values. (ii) Complex mortality, where rates of disease-induced mortality vary with both host sex and pathogen genotype. We use the maximum length of time that an infected individual could be tracked for each combination in the above experiment to calculate a constant mortality rate that would result in 99% of hosts dying by that maximum time and then convert this to a daily probability of dying, since we model the dynamics in discrete time. We also assumed that no transmission could happen after this point by setting mortality rates to NA (not applicable) beyond this time (which is why the average virulence, $\bar{v}(a)$ below, is not constant across the full range of infection days).

(c) Evolutionary dynamics

The above cross-infection experiment describes the within-host disease dynamics happening separately in males and females. In the electronic supplementary material, we develop an infection-age-structured model of environmental transmission (inspired by [46]), which allows us to make use of the theory developed in [30] to track the evolutionary dynamics of the mean transmission rate, $\bar{b}(a)$, and virulence, $\bar{v}(a)$, in the pathogen population. Throughout, an overbar represents an average across pathogen genotypes and a indexes the age of infection, in days. For each pathogen genotype, i , transmission at a given infection age is estimated as the composite function $b_i(a) = \phi \omega_i(a) v_i(a)$, since death is required for transmission; $\omega_i(a)$ is the infection-age and genotype specific spore load (see electronic supplementary material, table S1) and ϕ is a coefficient capturing the effective transmission rate of those spores (see derivation in electronic supplementary material). In all analyses, we arbitrarily set $\phi = 0.01$.

We investigate the evolutionary dynamics separately for each host sex, so the set of equations that follows are analysed independently for males and females. We also assume that hosts can only be infected by a single pathogen genotype. Because the data described above were collected daily, we use the discrete-time version of the evolutionary dynamics from [25], which are given by

$$\Delta \bar{b}(a) \approx \frac{1}{k} \left(S \sum_{s=0}^{\infty} q(s) G_{b,b}(a, s) - \sum_{s=0}^{\infty} q(s) \sigma(s) G_{b,v}(a, s) \right) \quad (2.1a)$$

and

$$\Delta \bar{v}(a) \approx \frac{1}{k} \left(S \sum_{s=0}^{\infty} q(s) G_{v,b}(a, s) - \sum_{s=0}^{\infty} q(s) \sigma(s) G_{v,v}(a, s) \right). \quad (2.1b)$$

Here, k is a combined measure of the generation time of infections and transmission [30], $q(s)$ describes the stable age distribution of infections, S is the density of susceptible hosts, and $\sigma(s)$ represents the reproductive value of an infection of age s . Expressions for k and $\sigma(s)$ are provided in the electronic supplementary material. $G_{b,b}(a,s)$ captures the genetic covariance between transmission rate at age a and at age s (and similarly $G_{v,v}(a,s)$ for virulence), while $G_{b,v}(a,s)$ and $G_{v,b}(a,s)$ describe the genetic cross-covariance between transmission and virulence at infection ages a and s . Note that for the simplified mortality scenario described above, virulence across pathogen genotypes for a given host sex is constant; thus we only analyse the evolutionary dynamics of transmission rate in this case and the second term in (2.1a) goes to 0.

The above equations make clear that the evolution of either trait is governed by direct selection acting on that trait at a given infection age, constraints due to genetic correlations in that trait across infection ages and indirect selection due to genetic correlations between traits. The first term in (2.1a) shows, for example, that (i) increased transmission is always favoured in a way that is proportional to the density of susceptible hosts, S , and (ii) evolutionary changes in transmission rate at any infection age, a , may also be constrained by genetic correlation with transmission rates at other infection ages, s , which are measured by the covariance matrix, $G_{b,b}(a,s)$. These effects are weighted by the proportion of all infections that are of age s , i.e. $q(s)$, and summed across all ages. The second term in (2.1a) captures the fact that virulence at infection age s can influence evolutionary change in the transmission rate at age a , given some genetic covariance between them, $G_{b,v}(a,s)$. These effects are again weighted by the proportion of all infections that are of age s , $q(s)$, but also by the reproductive value of those infections, $\sigma(s)$, since evolutionary changes in virulence will have consequences for the reproductive output of all future infection ages (since virulence ends infections). Similar arguments can be made for equation (2.1b).

(d) Epidemiological settings

To explore how the influence of male–female differences might depend on the type of epidemiological dynamics occurring in a population, we considered two scenarios: an expanding epidemic and an endemic situation. In an expanding epidemic, the number of infected hosts is increasing as is typical of a short-term disease outbreak. In contrast, in an endemic scenario the number of infected hosts eventually reaches a steady state and the pathogen persists in the population. To incorporate each scenario, we define a matrix ($\mathbf{L}$) of the transitions between infections of different ages, similar to a Leslie matrix, as in Mideo *et al.* [25], which allows different epidemiological scenarios to be explored without having to explicitly model the epidemiological dynamics. The details of this matrix are included in the electronic supplementary material. We treat each sex separately and so define sex-specific $\mathbf{L}$ matrices. The long-term growth rate of infected hosts (either male or female) is given by the dominant eigenvalue of this matrix (λ), and $q(s)$ is its associated eigenvector. We assume these dynamics occur fast relative to the speed of evolutionary change.

For the expanding epidemic, we arbitrarily set the number of susceptible hosts in the population, S , to 1000, and then calculate λ and $q(s)$ as the dominant eigenvalue and associated eigenvector of $\mathbf{L}$. For the endemic setting, we use the fact that the number of infected hosts reaches a stable equilibrium when the dominant eigenvalue of $\mathbf{L}$ is equal to 1. We find the value of S that satisfies this condition for each set of male and female estimates for average transmission rate ($\bar{b}(a)$) and virulence ($\bar{v}(a)$) across infection ages. We then calculate $q(s)$ as the eigenvector associated with $\lambda = 1$. For all simulations, S in the endemic case was between

40 and 140. With $q(s)$ and λ defined for both the epidemic and endemic epidemiological settings, we can calculate the last components of equations (2.1a) and (2.1b), k and $\sigma(s)$, and predict the resulting evolutionary dynamics using the sex-specific G matrices estimated from the data-derived transmission and mortality (virulence) rates (electronic supplementary material, tables S1 and S3). As a final step, we predict the rate of evolutionary change in our target trait of interest, $\bar{\omega}(a)$, the average spore loads at each age of infection in males and females, following equation (S8c) outlined in the electronic supplementary material.

3. Results

(a) Within-host dynamics for males and females

The within-host dynamics describing infections in males and females are shown in figure 1. For all combinations of pathogen genotype and host sex, the production of transmission spores increased significantly with the time since an infection occurred (see electronic supplementary material, table S1), and infections in females produced up to six times as many spores for pathogens at later ages than in males. Overall, the difference in spore production across male and female hosts appears to increase over the course of infection. However, the rate at which this increase occurred depended on a three-way interaction between host sex, pathogen genotype and the days post-infection (2-factor ANCOVA: $F_{5,1554} = 7.36$, $p < 0.001$; see electronic supplementary material, table S2 for the full model). At one extreme, for example, the rate of increase in spores over time was almost identical between males and females infected by pathogen C24 (females: slope = 0.133 ± 0.020 ; males: slope = 0.129 ± 0.015); whereas at the other end, for pathogen C01, infections in females reached far higher spore loads than in males for a given amount of time (females: slope = 0.143 ± 0.009 ; males: slope = 0.025 ± 0.004).

(b) Scenario 1: evolution of pathogen spore loads with simplified mortality

We first explored the evolutionary consequence of the contrasting male and female within-host dynamics under a simplified, yet biologically relevant [26,36], scenario where the rate at which infection ends is higher in males than in females (see electronic supplementary material, table S3). We found that the patterns of transmission at each infection age (figure 2a; calculated directly from the data as $\phi\omega_i(a)v$) and the resulting covariance within and between different infection ages (figure 2b) were qualitatively different between the two sexes. Transmission rates were generally higher in females across all infection ages, except for one pathogen genotype (C24; black lines), at very late ages of infection. This is driven by the steeper slope of spore loads in this genotype relative to the others (figure 1). The resulting covariance in transmission for female-limited infections were non-negative for almost all infection ages, except for one small region (yellow areas of figure 2b, top) and genetic variance (displayed along the diagonal) was lowest at intermediate infection ages. In contrast for the male-limited infections, there was a clear genetic trade-off in the form of negative covariance between the transmission rates at early and late ages (large yellow region in figure 2b, bottom), and a general increase in genetic variance as the infection age increased. Again, this pattern is driven by the much steeper

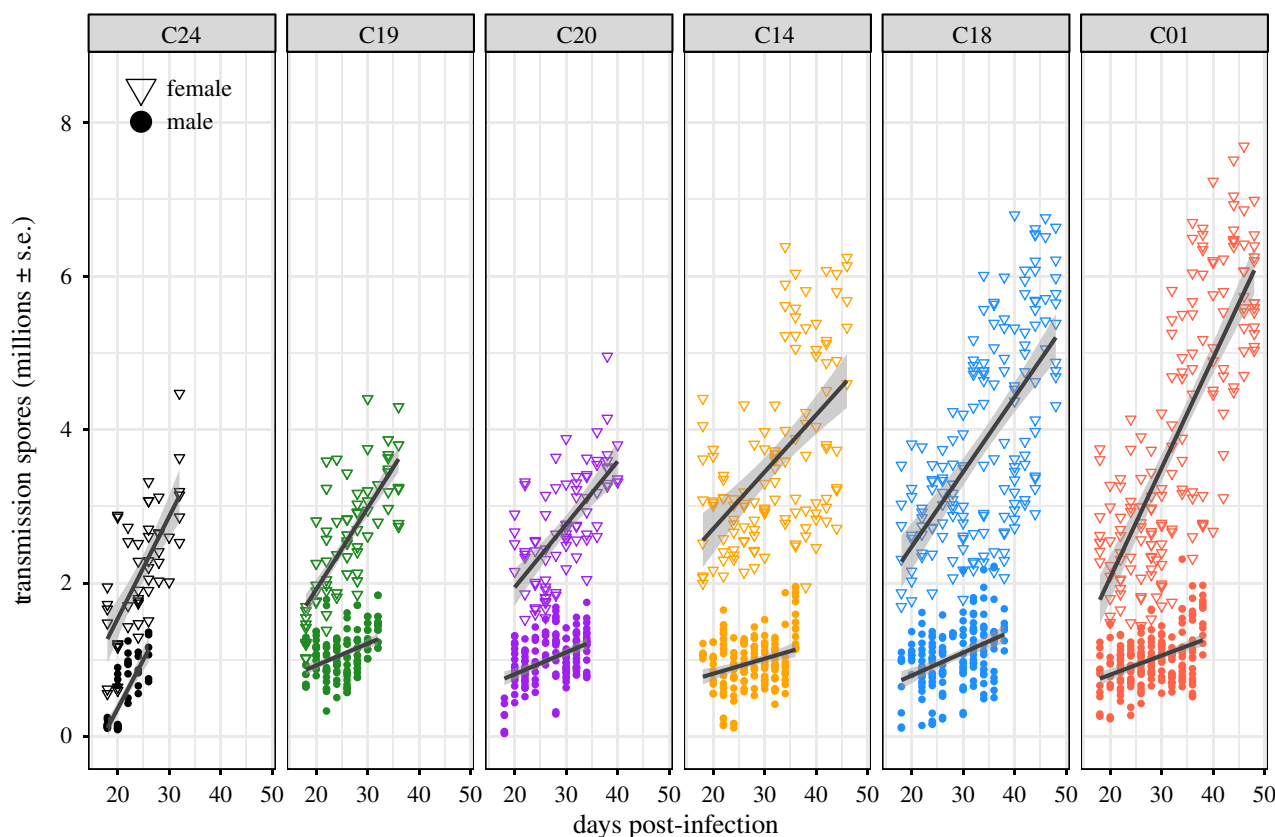


Figure 1. The dynamics of pathogen spore loads over the course of experimental infections in male (closed circles) and female (open triangles) *Daphnia*. Each panel presents a distinct genotype of the pathogen *P. ramosa*, arranged from left to right by increasing maximum spore loads achieved by the end of the experimental infections. Data presented are the raw spore loads of up to 20 infected animals destructively sampled every 2 days, beginning at day 18 post-infection and ending when the allotted pool of experimental animals was exhausted.

slope in spore loads (and lower intercept) for one genotype compared with the other genotypes.

The differences between infections limited to males or females as observed in the experiment, and quantified by the covariance matrices, extended to the evolutionary trajectory of average spore loads predicted by the model (figure 2c). In the absence of any trade-offs with virulence, both epidemic and endemic epidemiological settings generate selection for increased spore loads, but the relative importance of different ages of infection varied. In the epidemic case, in general, selection is particularly strong owing to the abundance of susceptible hosts, and favours early transmission due to the benefit this provides in an expanding outbreak (red lines, figure 2c). Not surprisingly then, for female-limited infections spore loads are predicted to increase across most ages, because of the general lack of any trade-offs among transmission rates, but with the greatest rate of change happening early in the infection. In contrast, for the male-limited infections, the trade-off between early and late transmission rates prevents spore loads from increasing across all ages. While spore loads are predicted to evolve upwards early in the infection, this comes at the expense of spore loads late in the infection.

Notably, the divergence in the evolutionary trajectory of spore loads under male-limited and female-limited infections is greatly reduced under the endemic setting (black lines, figure 2c). Here, pathogen spore loads remain relatively unchanged across all ages for female-limited infections and are only predicted to decline by a very small amount per day at later ages for male-limited infections. These results highlight that the extent to which sex differences could lead

to different evolutionary trajectories depends on the strength of selection and the relative importance of different infection ages. For the endemic setting, the number of susceptible hosts in a population is much smaller than when an epidemic arises, substantially reducing the strength of selection for increased transmission rates across all ages of infection. Late transmission also plays a more prevalent role, as the age distribution of infections is more uniform under this setting, disproportionately reducing the strength of selection for early transmission. As a consequence, the magnitude of any evolutionary response under male-limited and female-limited infections appears to be greatly reduced, even entirely absent, under the endemic case.

(c) Scenario 2: evolution of pathogen spore loads with complex mortality

We next explored the more complicated evolutionary scenario where the rate at which infection ends is higher in males than in females and depends on the genotype of *Pasteuria* that is infecting a given sex. Here, we used the maximum sampling duration that could be obtained in the above cross-infection experiment as a proxy for genotype-specific virulence in male-limited and female-limited infections (see electronic supplementary material, table S3). Adding this experimentally observed pathogen genetic variation in virulence alters the estimates of transmission rate (here, $\phi\omega_i(a)v_i(a)$), changes the covariance underlying the transmission trajectories and introduces cross-covariance—and consequently trade-offs—between transmission rates and virulence that can emerge at some ages and not others [25].

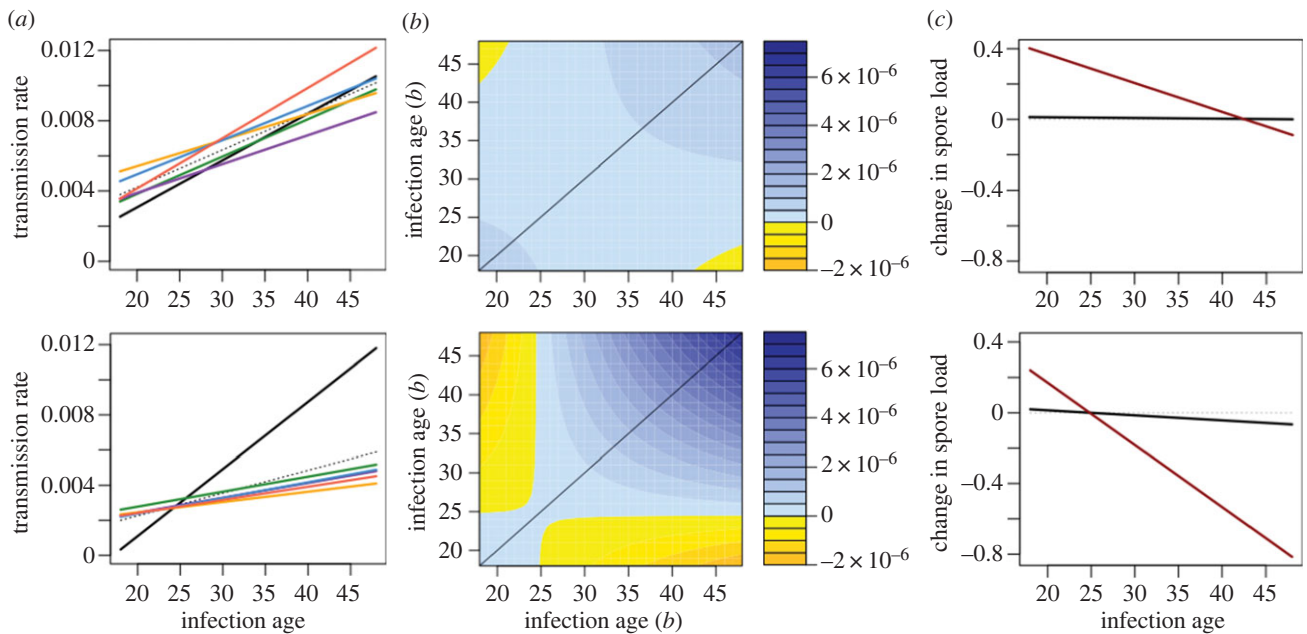


Figure 2. The evolution of pathogen spore loads under scenarios of simplified mortality, with predictions for females along the top row and males along the bottom row. (a) Pathogen transmission rates estimated by the composite transmission function $b_i(a)$. Colours for each pathogen genotype match that of figure 1, with the population average transmission rate indicated by the dashed line. (b) Patterns of covariance in transmission ($G_{b,b}$) resulting from the pathogen genotype-specific transmission trajectories. For each pair of infection ages (x – y combinations), blue represents positive covariance between transmission rates at those ages and yellow represents negative covariance between transmission rates at those ages. Values along the diagonal correspond to the genetic variance in transmission rate at a given age. For both (a) and (b) values are in units of per susceptible host per day. (c) Predicted rates of change in average spore loads. Predictions are shown for an epidemic (red lines) and endemic (black lines) setting. Actual values represent change per day in spore loads, with spore loads predicted to either increase (positive values, above dotted line) or decrease (negative values, below dotted line) at each infection age.

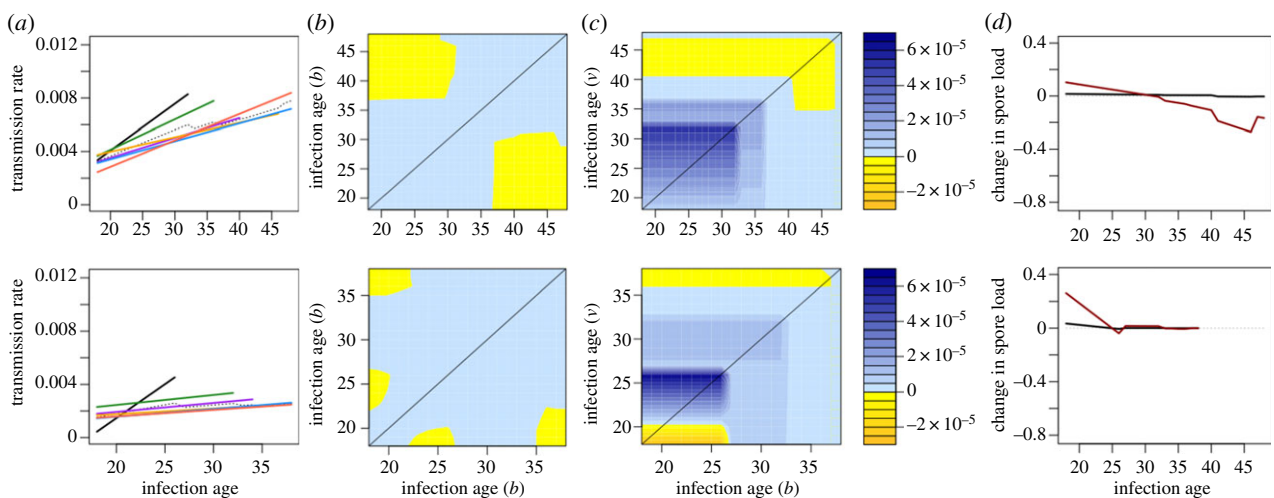


Figure 3. The evolution of pathogen spore loads under scenarios of complex mortality, with predictions for females along the top row and males along the bottom row. (a) Pathogen transmission rates estimated by the composite transmission function $b_i(a)$. Colours for each pathogen genotype match that of figure 1, with the population average transmission rate indicated by the dashed line. (b) Patterns of covariance in transmission ($G_{b,b}$) resulting from the genotype-specific transmission trajectories. (c) Patterns of cross-covariance between transmission and virulence ($G_{b,v}$). Values along the diagonal represent covariance between transmission rate and virulence at a given infection age, while values in the off-diagonals represent cross-covariance between transmission rate at one infection age (given along the x -axis) and virulence at another (given along the y -axis). As above, blue represents positive covariance between traits at those ages and yellow represents negative covariance between traits at those ages. (d) Predicted rates of change in average spore loads with red lines corresponding to the epidemic setting and black lines referring to the endemic setting. Actual values represent change per day in spore loads, with spore loads predicted to either increase (positive values) or decrease (negative values) at the different infection ages.

As with the scenario above, the transmission rates in females remained higher than in males at all ages of infection (figure 3a). The underlying covariance within and between different infection ages, however, was different. Females see an increase in the negative covariance between early and late transmission, and hence a stronger genetic trade-off,

but the reverse pattern is true for males (figure 3b). The cross-covariance between transmission and virulence was also mostly positive (figure 3c)—as expected since death is required for transmission—with some notable exceptions. In both sexes, virulence at late infection ages is negatively correlated with transmission across all infection ages, in part

driven by our assumption (for scenario 2) that no transmission can happen after the last infection day observed. Thus, fewer genotypes contribute to estimates at later ages and the genotypes that lead to the longest infections (contributing estimates of virulence at those late ages) tend to have lower than average transmission rates (e.g. red, blue and orange lines in figure 3a). In males only, transmission at early infection ages is also negatively correlated with virulence at early infection ages; arising because the genotype with the highest virulence in males (C24) also has the lowest early spore load (figure 1) and transmission rate (figure 3a, bottom; black lines).

The net effect of adding the countervailing cost of virulence (i.e. eliminating the possibility of future spore production) into our model of evolutionary dynamics is more moderate changes in spore loads, despite the benefit of terminating the infection for transmission. Under the epidemic setting, spore loads are predicted to increase at early infection ages in both male and female hosts (red lines, figure 3d), with a concomitant reduction in spore loads at late ages in females, owing to the negative covariance. As with the previous simplified scenario, the evolutionary responses are further reduced when the intensity of selection for transmission declines in the endemic setting (black lines, figure 3c).

4. Discussion

With sexual dimorphism occurring in the probability of infection, the production of spores within the host, rates of mortality and the duration of infection for many species [6–10], a pathogen's fitness appears intimately linked to the sex of its host. Yet the evolutionary consequences of these differences remain unclear (reviewed in [13]; but see [23]). By adapting a function-valued trait approach for studying the evolution of infectious disease life-histories [25,30], we explore how constraints arising from what happens to a pathogen within a male or female host can impact on the evolutionary trajectory of pathogen traits, such as the production of transmission spores. As a proof of concept for how sex differences might matter, we consider the two extreme possibilities that a pathogen could face in any species with two separate sexes—evolving completely through a series of male-limited or female-limited infections—and focus on the relative importance of within-host and between-host dynamics.

Our results highlight how the dynamics occurring within a host can be fundamentally different depending on whether a pathogen is infecting a male or female. On average, *Pasteuria* infections in female *Daphnia* hosts resulted in at least twice as many spores at a given age than the corresponding infection in a genetically identical male host (figure 1). This disparity between pathogen productivity in males and females only increased as the infection progressed, with a six-fold advantage occurring late in infections. With a pathogen's probability of establishing a new infection proportional to the release of spores at host death [44,45], this represents a substantial advantage to a pathogen able to infect a female over a male, either by chance or design. A single snapshot of pathogen performance may have failed to capture this degree of heterogeneity. For other disease systems, where pathogen density can peak at intermediate infection ages (e.g. malaria [47,48]), sex differences at this peak (or indeed, at other points in the infection) may only be captured by

exploring the trajectory of within-host dynamics in males and females.

On the basis of the within-host dynamics, females seem to be more evolutionarily liable for the pathogen, with higher spore loads and greater mean differences among pathogen genotypes as infection progresses (see also [34]). A within-host advantage to a pathogen, however, does not necessarily translate into elevated transmission between hosts (reviewed in [19]). While the transmission rates we estimated were generally higher in females across all ages of infection (figures 2a and 3a), mirroring the within-host growth trajectories of the pathogen genotypes, our epidemiological models predicted rates of evolutionary change of similar magnitude under male-limited and female-limited infections (figures 2c and 3d). Contributing strongly to this result was the nature of selection arising from transitions between susceptible and infected hosts at the population level. In an expanding epidemic, selection favours early transmission, reducing the advantage a pathogen might gain at later ages from a female-limited infection; while, for an endemic setting, selection may simply be too weak for even the fitness advantage of female-limited infections to generate change. Between-host transmission thus appears to offset the lower performance of a pathogen within a male host, making even subtle differences between the sexes evolutionarily relevant, as long as the selection generated by the between-host dynamics is sufficiently strong.

Our model shows how simple within-host processes occurring in males and females can lead to complex patterns of genetic constraint on pathogen evolution during an expanding epidemic (akin to pleiotropy as a source of genetic constraint [49,50]). Whenever negative covariance arose between transmission at different ages, as for males in scenario 1 or females in scenario 2, the resulting trade-off led to evolution favouring increased early spore loads at the expense of late. Conversely, when covariance was non-negative at most ages, spore loads were predicted to evolve upwards for all ages at which there was genetic variation available (scenario 2 for males and scenario 1 for females). Contributing to these differences are likely to be a variety of factors, including dimorphism in immune investment, or simply that males are smaller and less exploitable than females [26,36,37]. Notably, however, none of the predicted responses were concordant between male-limited and female-limited infections within a given mortality scenario. Instead, the effect of each sex was specific to whether mortality rates were higher in males (scenario 1) or varied with the genotype of the pathogen (scenario 2).

These findings provide evidence that differences between males and females in their vulnerability to infection can translate into divergent evolutionary trajectories for a pathogen. Such predictions, at face value, are most directly relevant for populations where one sex is often at higher abundance, such as those that are facultatively parthenogenetic, aggregate and form leks, or are maintained as livestock (i.e. essentially sex-limited like our model). Yet even in populations of *Daphnia*, which are predominantly female biased, males can reach up to half of the population for part of a season, making it highly probable that a pathogen will encounter both sexes each generation (see [40,51]). While our model shows how genetic constraints (from within-host dynamics) and patterns of selection (from between host dynamics) can arise differently when infections pass through

either male or female hosts, it cannot predict the evolution of a pathogen once it encounters both males and females. Theory on the evolution of optimal host exploitation in two sexes [23], however, suggests that with limited between-sex transmission and equal sex-ratios, overall pathogen evolution should track the trajectory imposed by the least resistant host (e.g. females in *Daphnia*, see discussion in [26]). Alternatively, as between-sex transmission increases, pathogen evolution will potentially be forced into an outcome that is intermediate to the trajectory predicted by each sex in isolation (as per broader theory on host heterogeneity, [12]). Incorporating fluctuations in the relative numbers of males and females within a population into our function-valued trait approach will help establish the conditions that see a pathogen pulled away from the extreme evolutionary trajectories that infecting each sex intrinsically defines.

More precise estimates of age-specific mortality (e.g. [26,52]) and sex-specific infection rates would further refine our predictions and additionally open up more nuanced aspects of the transmission-virulence trade-off (e.g. [53,54]) to be partitioned by sex. For both sexes, increasing the complexity of mortality in the model reduced the magnitude of any evolutionary response. Introducing the countervailing cost of virulence (e.g. second term in equation (2.1a)) enabled trade-offs between transmission and virulence that either further constrained pathogen evolution or disrupted genetic variation at later ages via the selective disappearance of certain genotypes. While our characterization of within-host growth is comprehensive, the estimates we used for genotype-specific mortality rates, while concordant with other studies in this system [26,36], are only a proxy for the true age-specific mortality rates that each pathogen would induce in males and females. Similarly, estimates of infectiousness in both male and female hosts for different pathogen genotypes or strains would introduce additional genetic constraints on pathogen evolution. These are integral

components of current models exploring the optimal strategy of a pathogen in male and female hosts [23,24], but have yet to be integrated with a quantitative genetic approach to understanding pathogen evolution.

In summary, our model thus serves a broader purpose in demonstrating how genetic constraints underlying pathogen evolution can arise differently in males and females, and reiterates the importance of viewing pathogen performance in light of between-host dynamics. This work sets the stage for simple changes in the relative number of males and females in a population, or the rate at which a pathogen is transmitted between the different sexes, to become keenly relevant for the evolution of a pathogen (see also [23,24]; and theory on host heterogeneity, e.g. [11,12]). Incorporating the likelihood of a pathogen encountering males and females at each transmission event, as well as sex-specific susceptibility, is the logical extension of our function-valued approach, and one that holds great promise for understanding the importance of male–female differences at both the within-host and between-host scales.

Data accessibility. The electronic supplementary material contains details of the model of between-host dynamics and the results of the statistical analysis describing the within-host dynamics of infection in male and female *Daphnia*. Data for this publication are available online via a Dryad Digital Repository. (<https://doi.org/10.5061/dryad.s7n1b44>).

Authors' contributions. M.D.H. designed the study and performed the analysis. N.M. developed and integrated the epidemiological model. M.D.H. and N.M. wrote and edited the manuscript. All authors gave final approval for publication.

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References

- Andersson MB. 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.
- Allen SL, Bonduriansky R, Chenoweth SF. 2018 Genetic constraints on microevolutionary divergence of sex-biased gene expression. *Phil. Trans. R. Soc. B* **373**, 20170427. (doi:10.1098/rsth.2017.0427)
- De Lisle SP, Goedert D, Reedy AM, Svensson EI. 2018 Climatic factors and species range position predict sexually antagonistic selection across taxa. *Phil. Trans. R. Soc. B* **373**, 20170415. (doi:10.1098/rsth.2017.0415)
- Mishra A, Tung S, Shreenidhi PM, Aamir Sadiq M, Shree Sruti VR, Chakraborty PP, Dey S. 2018 Sex differences in dispersal syndrome are modulated by environment and evolution. *Phil. Trans. R. Soc. B* **373**, 20170428. (doi:10.1098/rsth.2017.0428)
- Zuk M. 2009 The sicker sex. *PLoS Pathog.* **5**, e1000267. (doi:10.1371/journal.ppat.1000267)
- Zuk M, McKean KA. 1996 Sex differences in parasite infections: patterns and processes. *Int. J. Parasitol.* **26**, 1009–1023. (doi:10.1016/S0020-7519(96)80001-4)
- Schalk G, Forbcs MR, Schalk G, Forbes MR. 1997 Male biases in parasitism of mammals: effects of study type, host age, and parasite taxon. *Oikos* **78**, 67–74. (doi:10.2307/3545801)
- Poulin R. 1996 Sexual inequalities in helminth infections: a cost of being a male? *Am. Nat.* **147**, 287–295. (doi:10.1086/285851)
- McCurdy DG, Shutler D, Mullie A, Forbes MR. 1998 Sex-biased parasitism of avian hosts: relations to blood parasite taxon and mating system. *Oikos* **82**, 303–312. (doi:10.2307/3546970)
- Sheridan LAD, Poulin R, Ward DF, Zuk M. 2000 Sex differences in parasitic infections among arthropod hosts: is there a male bias? *Oikos* **88**, 327–334. (doi:10.1034/j.1600-0706.2000.880211.x)
- Osnas EE, Dobson AP. 2012 Evolution of virulence in heterogeneous host communities under multiple trade-offs. *Evolution* **66**, 391–401. (doi:10.1111/j.1558-5646.2011.01461.x)
- Gandon S. 2004 Evolution of multihost parasites. *Evolution* **58**, 455–469. (doi:10.1111/j.0014-3820.2004.tb01669.x)
- Gipson SAY, Hall MD. 2016 The evolution of sexual dimorphism and its potential impact on host-pathogen coevolution. *Evolution* **70**, 959–968. (doi:10.1111/evo.12922)
- Hall MD, Bento G, Ebert D. 2017 The evolutionary consequences of stepwise infection processes. *Trends Ecol. Evol.* **32**, 612–623. (doi:10.1016/j.tree.2017.05.009)
- Schmid-Hempel P. 2009 Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. *Phil. Trans. R. Soc. B* **364**, 85–98. (doi:10.1098/rsth.2008.0157)
- Alizon S, de Roode JC, Michalakakis Y. 2013 Multiple infections and the evolution of virulence. *Ecol. Lett.* **16**, 556–567. (doi:10.1111/ele.12076)
- Anderson RM, May RM. 1991 *Infectious diseases of humans: dynamics and control*. Oxford, UK: Oxford University Press.

18. Grenfell B, Harwood J. 1997 (Meta)population dynamics of infectious diseases. *Trends Ecol. Evol.* **12**, 395–399. (doi:10.1016/S0169-5347(97)01174-9)
19. Mideo N, Alizon S, Day T. 2008 Linking within- and between-host dynamics in the evolutionary epidemiology of infectious diseases. *Trends Ecol. Evol.* **23**, 511–517. (doi:10.1016/j.tree.2008.05.009)
20. Coombs D, Gilchrist MA, Ball CL. 2007 Evaluating the importance of within- and between-host selection pressures on the evolution of chronic pathogens. *Theor. Popul. Biol.* **72**, 576–591. (doi:10.1016/j.tpb.2007.08.005)
21. Nowak MA, May RM. 1994 Superinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B* **255**, 81–89. (doi:10.1098/rspb.1994.0012)
22. Stoehr AM, Kokko H. 2006 Sexual dimorphism in immunocompetence: what does life-history theory predict? *Behav. Ecol.* **17**, 751–756. (doi:10.1093/beheco/ark018)
23. Cousineau SV, Alizon S. 2014 Parasite evolution in response to sex-based host heterogeneity in resistance and tolerance. *J. Evol. Biol.* **27**, 2753–2766. (doi:10.1111/jeb.12541)
24. Ubeda F, Jansen VAA. 2016 The evolution of sex-specific virulence in infectious diseases. *Nat. Commun.* **7**, 13849. (doi:10.1038/ncomms13849)
25. Mideo N, Nelson WA, Reece SE, Bell AS, Read AF, Day T. 2011 Bridging scales in the evolution of infectious disease life histories: application. *Evolution* **65**, 3298–3310. (doi:10.1111/j.1558-5646.2011.01382.x)
26. Gipson SAY, Hall MD. 2018 Interactions between host sex and age of exposure modify the virulence-transmission trade-off. *J. Evol. Biol.* **31**, 428–437. (doi:10.1111/jeb.13237)
27. Sasaki A, Iwasa Y. 1991 Optimal growth schedule of pathogens within a host: switching between lytic and latent cycles. *Theor. Popul. Biol.* **39**, 201–239. (doi:10.1016/0040-5809(91)90036-F)
28. Alizon S, van Baalen M. 2005 Emergence of a convex trade-off between transmission and virulence. *Am. Nat.* **165**, E155–E167. (doi:10.1086/430053)
29. Gilchrist MA, Coombs D. 2006 Evolution of virulence: interdependence, constraints, and selection using nested models. *Theor. Popul. Biol.* **69**, 145–153. (doi:10.1016/j.tpb.2005.07.002)
30. Day T, Alizon S, Mideo N. 2011 Bridging scales in the evolution of infectious disease life histories: theory. *Evolution* **65**, 3448–3461. (doi:10.1111/j.1558-5646.2011.01394.x)
31. Stinchcombe JR, Function-valued Traits Working Group, Kirkpatrick M. 2012 Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends Ecol. Evol.* **27**, 637–647. (doi:10.1016/j.tree.2012.07.002)
32. Ebert D, Duneau D, Hall MD, Luijckx P, Andras JP, Du Pasquier L, Ben Ami F. 2016 A population biology perspective on the stepwise infection process of the bacterial pathogen *Pasteuria ramosa* in *Daphnia*. *Adv. Parasitol.* **91**, 265–310. (doi:10.1016/bs.apar.2015.10.001)
33. Hall MD, Ebert D. 2012 Disentangling the influence of parasite genotype, host genotype and maternal environment on different stages of bacterial infection in *Daphnia magna*. *Proc. R. Soc. B* **279**, 3176–3183. (doi:10.1098/rspb.2012.0509)
34. Clerc M, Ebert D, Hall MD. 2015 Expression of parasite genetic variation changes over the course of infection: implications of within-host dynamics for the evolution of virulence. *Proc. R. Soc. B* **282**, 20142820. (doi:10.1098/rspb.2014.2820)
35. Ebert D, Joachim Carius H, Little T, Decaestecker E. 2004 The evolution of virulence when parasites cause host castration and gigantism. *Am. Nat.* **164**, S19–S32. (doi:10.1086/424606)
36. Thompson O, Gipson SAY, Hall MD. 2017 The impact of host sex on the outcome of co-infection. *Sci. Rep.* **7**, 910. (doi:10.1038/s41598-017-00835-z)
37. Duneau D, Luijckx P, Ruder LF, Ebert D. 2012 Sex-specific effects of a parasite evolving in a female-biased host population. *BMC Biol.* **10**, 104. (doi:10.1186/1741-7007-10-104)
38. Roulin AC, Routtu J, Hall MD, Janicke T, Colson I, Haag CR, Ebert D. 2013 Local adaptation of sex induction in a facultative sexual crustacean: insights from QTL mapping and natural populations of *Daphnia magna*. *Mol. Ecol.* **22**, 3567–3579. (doi:10.1111/mec.12308)
39. Lampert W. 2011 *Daphnia: development of a model organism in ecology and evolution*. Oldendorf/Luhe, Germany: International Ecology Institute.
40. Galimov Y, Walser B, Haag CR. 2011 Frequency and inheritance of non-male producing clones in *Daphnia magna*: evolution towards sex specialization in a cyclical parthenogen? *J. Evol. Biol.* **24**, 1572–1583. (doi:10.1111/j.1420-9101.2011.02288.x)
41. Luijckx P, Ben Ami F, Mouton L, Du Pasquier L, Ebert D. 2010 Cloning of the unculturable parasite *Pasteuria ramosa* and its *Daphnia* host reveals extreme genotype–genotype interactions. *Ecol. Lett.* **14**, 125–131. (doi:10.1111/j.1461-0248.2010.01561.x)
42. Klüttgen B, Dülmer U, Engels M, Ratte HT. 1994 ADaM, an artificial freshwater for the culture of zooplankton. *Water Res.* **28**, 743–746. (doi:10.1016/0043-1354(94)90157-0)
43. Ebert D, Zschokke-Rohringer CD, Carius HJ. 1998 Within-and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*. *Proc. R. Soc. Lond. B* **265**, 2127–2134. (doi:10.1098/rspb.1998.0549)
44. Ben Ami F, Regoes RR, Ebert D. 2008 A quantitative test of the relationship between parasite dose and infection probability across different host-parasite combinations. *Proc. R. Soc. B* **275**, 853–859. (doi:10.1098/rspb.2007.1544)
45. Regoes RR, Hottinger JW, Sygnarski L, Ebert D. 2003 The infection rate of *Daphnia magna* by *Pasteuria ramosa* conforms with the mass-action principle. *Epidemiol. Infect.* **131**, 957–966. (doi:10.1017/S0950268803008793)
46. Day T. 2002 Virulence evolution via host exploitation and toxin production in spore-producing pathogens. *Ecol. Lett.* **5**, 471–476. (doi:10.1046/j.1461-0248.2002.00342.x)
47. Reece SE, Drew DR, Gardner A. 2008 Sex ratio adjustment and kin discrimination in malaria parasites. *Nature* **453**, 609–614. (doi:10.1038/nature06954)
48. Bell AS, de Roode JC, Sim D, Read AF. 2006 Within-host competition in genetically diverse malaria infections: parasite virulence and competitive success. *Evolution* **60**, 1358–1371. (doi:10.1111/j.0014-3820.2006.tb01215.x)
49. Connallon T, Hall MD. 2018 Genetic constraints on adaptation: a theoretical primer for the genomics era. *Ann. N. Y. Acad. Sci.* **1422**, 65–87. (doi:10.1111/nyas.13536)
50. Blows MW, Hoffmann AA. 2005 A reassessment of genetic limits to evolutionary change. *Ecology* **86**, 1371–1384. (doi:10.1890/04-1209)
51. Duncan AB, Mitchell SE, Little TJ. 2006 Parasite-mediated selection and the role of sex and diapause in *Daphnia*. *J. Evol. Biol.* **19**, 1183–1189. (doi:10.1111/j.1420-9101.2006.01085.x)
52. McDonald JL, Smith GC, McDonald RA, Delahay RJ, Hodgson D. 2014 Mortality trajectory analysis reveals the drivers of sex-specific epidemiology in natural wildlife-disease interactions. *Proc. R. Soc. B* **281**, 20140526. (doi:10.1098/rspb.2014.0526)
53. Ewald PW. 1983 Host-parasite relations, vectors, and the evolution of disease severity. *Annu. Rev. Ecol. Syst.* **14**, 465–485. (doi:10.1146/annurev.es.14.110183.002341)
54. Anderson RM, May RM. 1982 Coevolution of hosts and parasites. *Parasitology* **85**, 411. (doi:10.1017/S0031182000055360)