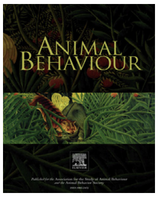
Animal Behaviour 107 (2015) 41–48

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



Assessing the impact of male-killing bacteria on the spread of a sexually transmitted infection

Daria Pastok, David Atkinson, Gregory D. D. Hurst*

Institute of Integrative Biology, University of Liverpool, Liverpool, U.K.

ARTICLE INFO

Article history:
Received 4 March 2015
Initial acceptance 30 March 2015
Final acceptance 26 May 2015
Published online

MS. number: 15-00171

Keywords:
epidemiology
mating system
sex ratio bias
sexually transmitted infection
symbiont

Theory predicts that the epidemiology of sexually transmitted infections (STIs) will be affected by any factor that alters the mating biology of its host. In insects, the presence of sex ratio-distorting symbionts may modulate population sex ratio, altering male and female encounter rates and creating the potential for symbionts to influence STI dynamics. We used laboratory experiments to investigate how a heritable male-killing *Spiroplasma* bacterial symbiont will affect the epidemiology of a sexually transmitted mite, *Coccipolipus hippodamiae* in the two-spot ladybird, *Adalia bipunctata*. We compared mating biology and STI transmission under 1:1 and 4:1 population sex ratios, equivalent to no male-killer presence and 75% male-killer prevalence. We observed that males have the potential to mate with enough females to maintain high female mating rate under strongly female-biased population sex ratios. Further, we observed that females remate more readily when males mate frequently, suggesting female mating rate may in fact be raised in female-biased populations. From the mite's perspective, these effects are countered, at least partly, by reductions in mite transmission from recently mated infected males. We modelled the impact of these processes on STI epidemiology, and concluded that strong sex ratio biases associated with male-killing bacteria are likely to speed the spread of STI infection through both male and female hosts. Thus, the presence of the male-killing bacterium is likely to have indirect impacts on female hosts, mediated through the STI.

Sexually transmitted infections (STI) are common in animal species in which at least one sex mates multiply (Knell & Webberley, 2004; Lockhart, Thrall, & Antonovics, 1996). The dynamics of STIs are often considered as distinct from that of other infections, as transmission rates are related to the host mating system, rather than more general patterns of movement. STI epidemiology depends on the mean number of partners with which an individual mates (promiscuity), the variance in mating success among individuals of a particular sex and the contact structure of matings (i.e. whether more promiscuous individuals tend to pair together; Ashby & Gupta, 2013; Thrall & Antonovics, 1997; Thrall, Antonovics, & Bever, 1997; Thrall, Antonovics, & Dobson, 2000). For instance, high variance in male reproductive success associated with male–male competition for mates produces female-biased epidemics of STIs (Ashby & Gupta, 2013; Nahrung & Clarke, 2007). To understand how STI epidemiology varies, therefore, we must understand the factors driving mating system variation.

One ecological factor known to affect mating systems is population sex ratio (Rankin & Kokko, 2007). In insects, sex ratio-distorting microbes represent important drivers of population sex ratio, which then alter the mating system of their host (Charlat et al., 2007; Hurst, Hurst, & Majerus, 1997; Jiggins, Hurst, & Majerus, 2000). The alteration in mating system creates a context in which the presence of a sex ratio-distorting microbe may impact upon the dynamics of an STI. One intuitive outcome is that males will on average mate more often than females in sex ratio-biased populations. This creates the prediction that STI epidemics should switch to being male-biased under female-biased population sex ratios (Hurst et al., 1997).

Recent work has established male-biased patterns of STI spread in natural populations of the two-spot ladybird, *Adalia bipunctata* (Ryder et al., 2014). This species is host to the mite *Coccipolipus hippodamiae*, which transfers between host individuals while they copulate, and thus is an STI. The ladybird also carries a variety of male-killing bacteria (Hurst, Jiggins, et al., 1999; Hurst, Von Schulenburg, et al., 1999), which locally reach high prevalence and can cause strongly female-biased populaton sex ratios. In Stockholm, Sweden, 70% of females carry a male-killing infection, producing a population sex ratio bias of four females per male. In

---

* Correspondence: G. D. D. Hurst, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, U.K.

E-mail address: g.hurst@liv.ac.uk (G. D. D. Hurst).

http://dx.doi.org/10.1016/j.anbehav.2015.06.002

this population, males mate more frequently than females, resulting in higher exposure to the STI. Here, male-biased epidemics of the STI are observed, in which male beetles become infected with mites on average 10 days before female beetles. In contrast, in areas where the *Spiroplasma* is rare, epidemic spread of the STI occurs at equivalent rates in male and female hosts (Ryder et al., 2014).

While the gross impact of population sex ratio bias on STI dynamics (male-biased epidemics) is evident in *Adalia*, we only have a very basic understanding of how STI epidemiology is altered by male-killer presence/sex ratio skew. Sex ratio skew has obvious impacts on the relative mating rate of males and females, predicting the observed male-biased epidemics. However, the impact of sex ratio bias on the speed of STI spread depends on how a female-biased sex ratio affects the absolute mating rate of the two sexes, which is at present unknown. There are three scenarios.

(1) Male mating rate shows low plasticity, and female mating rate thus declines because female mating becomes limited by access to available males. This scenario is most likely where males invest heavily in paternal care, for instance through provision of a nutritious spermatophore. Constraints on male mating rate would create an epidemic mildly slowed on male hosts (associated with lack of infection of female partners), and greatly slowed on females.

(2) Male mating rate shows sufficient plasticity that female mating rate remains the same in female-biased populations, and male mating rate elevates above the rate seen in a 1:1 population due to the increased availability of females. This would create an epidemic greatly accelerated on male hosts, and an epidemic in female hosts that was initially like that in a 1:1 population, but with later acceleration following the epidemic through male hosts.

(3) Male mating rate shows high plasticity as in scenario 2, but female mating rate also increases where males are rare. In *Hypolimnas* butterflies, for instance, female remating rate is higher in populations in which male-killing *Wolbachia* are common and the population is very female biased. The increase in female mating rate is a result of males with high remating rate not transferring sufficient spermatophore material to inhibit their female partners from future remating (Charlat et al., 2007). In ladybirds, female remating is likewise delayed as a consequence of spermatophore consumption (Perry & Rowe, 2008). It is possible that the resistance to remating associated with spermatophore transfer is diminished when males mate frequently, and transfer either fewer or smaller spermatophores. If this were the case, then we would predict that a female-biased sex ratio would be associated with acceleration of the STI epidemic on female hosts because of reduced inhibition of their remating rates.

The above describes the potential impact of sex ratio distortion on mating rate. However, the epidemiology of the STI is a function of both mating rate and the probability of STI transmission during sexual contact. It is thus important to also ascertain whether high mating rates affect the chance of STI transmission during copulation. In the ladybird system, new infections are produced by the transfer of motile larval mites (Hurst et al., 1995). An infected host will produce a limited number of larval mites each day (one mature adult mite lays one to three eggs per day; wild female ladybirds carry one to six mature female mites). While larval mite supply is sufficient to create very high per copulation transmission efficiency at low mating rates (Hurst et al., 1995; Ryder et al., 2014), a rapid remating rate may diminish the 'pool' of larval mites waiting to transfer, impacting on the probability of mite acquisition following copulation. This would then buffer the population against increased transmission opportunity associated with a biased sex ratio.

The impact of sex ratio on mating biology and mite transmission is not easily gauged in the field, as populations that differ in sex ratio also differ in a wide variety of other factors that affect mating rate (e.g. temperature, food: Ryder et al., 2013). We therefore used laboratory experiments to ascertain the likely impact of female-biased population sex ratios on the epidemiology of *C. hippodamiae* on *A. bipunctata*, and then used these data to inform a mathematical model to explore how these various aspects interact to drive STI epidemiology. We examined first the degree of plasticity of male mating rate, and the impact of this on per contact mite transmission rates. We emulated a four female:one male population sex ratio bias, and tested whether a male that is offered females at four times the 'natural' rate achieves four times as many copulations, and whether a male that mates frequently is as likely to transfer infection to his partner. Second, we examined whether female propensity to remate is increased if males have a recent history of mating activity. Finally, we explored the epidemiological consequences of our experiments by modifying an existing model of *C. hippodamiae* dynamics.

METHODS

Newly eclosed adult *A. bipunctata* were collected in Stockholm in August 2013 to produce our experimental generation. These newly emerged adults were all uninfected, as they had no opportunity to mate with infected older adults. We also collected some older infected females, and used them to infect some of the adult males of the experimental generation. Mite infection development was monitored, and males used in the experiment were those that had developed 5–30 larval mites, implying a mature infection, but not one made artificially intense through lack of mating (mite infection does not affect male mating success in this species: Webberley, Hurst, Buszko, & Majerus, 2002). Females for experiments were uninfected in all cases, and either collected from the field as newly generated adults, or bred in the laboratory and matured for 20 days. Virgin females were used where possible. Where female reuse was necessary, the female had not mated in the previous 7 days, which restores female desire to mate to a high level (Haddrill, Shuker, Mayes, & Majerus, 2007).

Experiment 1: Plasticity of Male Mating Rate

Methods

In this experiment, we asked whether males can maintain a high mating rate with increased exposure to females, and whether mite transmission is affected by male mating rate. Sixty male ladybirds that were infectious for mites were mated 3 days prior to the experiment, and then offered four females in sequence in two mate exposure treatments. In the 'standard' mating opportunity treatment, males were offered an opportunity to mate every 2 days (in the morning), with the four mating opportunities therefore spread over 6 days. This rate (once every 2 days) is the equivalent of that achieved by a male during the spring mating system in a 1:1 sex ratio population (Haddrill, Shuker, Amos, Majerus, & Mayes, 2008). Female partners were virgin and mite free. In the 'frequent' mating opportunity treatment, males were offered females in the morning and in the evening for 2 days. This emulated a population with a four female:one male sex ratio in which males had females offered at four times the rate normally available.

Each mating opportunity lasted 1 h, and the presence/absence of mating was noted. When mating occurred, beetles were placed in an incubator at 22 °C and allowed to finish mating. We recorded the mating duration before separating the ladybirds and putting them in individual petri dishes. A fixed temperature for completion of mating was used, as mating duration is thermally sensitive. Where mating occurred, the recipient females were then maintained at 22 °C to ensure consistency of treatment (mite latent period is thermally sensitive). The number of larval mites on the