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Constraints on parasite fecundity and transmission in an insect-STD system

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Parasites that are sexually transmitted (causing sexually transmitted diseases, or STDs) can have important effects on host population dynamics, but we know almost nothing for such parasites about constraints on fecundity and transmission. In this study, we have examined the effect of two potentially important constraints in one of the few empirically well-studied animal-STD systems, the ladybird, *Adalia bipunctata*, and its sexually transmitted mite, *Coccipolipus hippodamiae*. Using a factorial design, we manipulated: (i) within-host competition, by varying infection intensity; and (ii) host condition, by introducing dietary stress. Infection with *C. hippodamiae* significantly reduced ladybird survival whether or not diet was restricted, and restricting diet led to reduced survival regardless of infection status. Increased infection intensity and reduced host condition (dietary stress) both independently constrained per capita rates of parasite egg production and the development of infective larvae. Furthermore, when host condition was compromised, significantly fewer larvae were transmitted per adult mite during copulation. The effect of infection intensity on per mite transmission was more complex: there was no significant effect when hosts were fed normally, but when the ladybirds were nutritionally stressed higher infection intensity was associated with a slight increase in the numbers that were transmitted (despite the fact that mite fecundity was reduced under these conditions). These results indicate that host condition and within-host competition may both play an important role in shaping the epidemiology of the *A. bipunctata*–*C. hippodamiae* system, by influencing the parasite's basic reproductive ratio (R_0) and the rate of epidemic spread. Our data also extend general insights into STD ecology, by highlighting the importance of constraints on disease dynamics that are likely to be widespread.

Parasites can have a fundamental influence on the population dynamics of their hosts (Tompkins et al. 2002). Recent empirical and theoretical developments have advanced our understanding on many fronts, but progress has been slower for parasites that are primarily sexually transmitted (causing sexually transmitted diseases, or STDs). Experimental data, in particular, are lacking for animal-STD systems and as a result we undoubtedly underestimate their ecological significance (Lockhart et al. 1996, Knell and Webberley 2004).

In any host ~ parasite system, the transmission of infection is the most fundamental step. For microparasites (many STDs are caused by microparasites), population models tend to describe this process in terms of both the rate of contact between infected and susceptible hosts and the per-contact probability of transmission (Begon et al. 2002). Contact rates will largely depend on host demography and behaviour, whereas transmission probabilities will vary with a variety of factors that are specific to the particular combination of host and parasite. For convenience, both of these components are usually treated as constants and combined into a single transmission coefficient (typically β). More realistically, it is known that density-dependent variation in contact rates can cause empirical estimates of β to vary (Knell et al. 1998). Similarly, per-contact transmission probabilities are unlikely to be fixed. For example, parasite reproductive rate may be constrained by within-host competition in both micro- and macroparasites (Hudson and Dobson 1997, Keas and Esch 1997, Tripet and Richner 1999, Ebert et al. 2000, Irvine et al. 2001, Bedhomme

et al. 2004, Logan et al. 2005). However, despite the potential impact on transmission dynamics, we know of no studies that have explicitly linked constraints on parasite fecundity with transmission.

The ectoparastic mite, *Coccipolipus hippodamiae*, is a common parasite in many European populations of its insect host, the two-spot ladybird, *Adalia bipunctata* (Webberley et al. 2004, Webberley et al. 2006b). The adult mites are sessile and attach to the underside of the beetle's wing-cases, where they feed on haemolymph. Mite eggs hatch after approximately seven days of incubation (at 23°C) and give rise to motile infective larvae, which transfer to another host when the beetles mate. Transmission is almost exclusively sexual. Non-sexual transmission can only occur when the beetles are maintained in very close proximity (Hurst et al. 1995) and then only occurs at low levels, during the over-wintering period when the beetles often aggregate (Webberley and Hurst 2002). *C. hippodamiae* is also highly virulent. The mites induce sterility among female ladybirds soon after infection (Hurst et al. 1995) and also cause an increase in over-wintering mortality (Webberley and Hurst 2002). Surprisingly, *A. bipunctata* seem to lack an effective immune response to *C. hippodamiae*: almost all beetles that acquire infective larvae become infected and recovery rates are very low.

In epidemiological terms, *C. hippodamiae* is probably best modelled as a microparasite (May and Anderson 1979, Ryder et al. 2005). It has a high rate of direct reproduction on its host and its basic reproductive ratio, R_0, can defined in terms of the number of new infections that an infectious individual would cause in a population of susceptible hosts. Nevertheless, the adult mites are quite large and may consume a substantial amount of their host's resources. An infected beetle may carry, for example, 30 adult mites, but probably not many more. Therefore, given that the mites acquire all of their nutritional resources from their host's haemolymph, it seems likely that mite fecundity will vary with the degree of competition among mites infecting the same beetle and with host condition. Both types of constraint should influence transmission. For example, infected beetles may be intermittently non-infectious if mite fecundity is low, as repeated transmission events may exhaust the pool of infective larvae. Previous work has established that *A. bipunctata* mate once every two or three days at the height of reproductive activity during the summer months (Webberley et al. 2006a), making such an effect possible. Reduced fecundity may also impact on mite behaviour and transmission efficiency, if there is an associated change in the allocation of resources to eggs, influencing embryonic development. Similar effects have been reported in other insect parasites, such as entomopathogenic nematodes (Ryder and Griffin 2002, 2003). Either of these outcomes may influence the dynamics of the system, by reducing the per-contact probability of transmission and, consequently, infection rates.

Within-host constraints on parasite fecundity (i.e. within-host competition and host condition) are potentially of considerable epidemiological significance, but we know of no other study that has experimentally examined their impact on transmission. Such data is particularly valuable in the context of STDs, given the current dearth of experimental data on STD dynamics. The objectives of the present study, therefore, were (a) to determine if increased within-host competition and/or reduced host condition constrain parasite fecundity in the *C. hippodamiae*–*A. bipunctata* system; and (b) to measure the effect on transmission. To manipulate within-host competition, we varied infection intensity by experimentally infecting ladybirds with different numbers of infective mite larvae. We manipulated host condition by restricting the diet of control and infected beetles and monitoring the effect on survival rates.

Material and methods

Experimental infection

We collected infected and uninfected *Adalia bipunctata* from various locations in Berlin, Germany, in August 2004 and used them to found laboratory cultures in London (*Coccipolipus hippodamiae* is not currently known to infect *A. bipunctata* in the UK). Details of the culturing protocol have been published previously (Ryder et al. 2005). Field-collected adult ladybirds were used to infect the first generation of lab-reared, uninfected adults. We infected adult females artificially by using a fine piece of nylon to transfer infective larvae and/or mite eggs to the undersides of their wing-cases, between 12 and 20 days post-emergence from the pupal stage. Uninfected, control females were given a sham infection, which involved manipulating the wing-cases in the same way and at the same time as the infected females. Males were only used to measure transmission (i.e. from females to males following copulation; see below) and not in the assessment of mite fecundity (although the mites develop equally well on both sexes).

Following pupation of the first generation of lab-reared ladybirds, we randomly assigned adult females to one of six different treatments. Half the ladybirds were given access to a 'normal', unrestricted diet of pea aphids, *Acyrthosiphon pisum*, whilst the remaining half were given restricted access to food, with aphids being provided on one out of every three days, creating a nutritionally stressed, 'low' food treatment. Within each of these two groups, ladybirds were either infected with 10 or 4 infective mite larvae, or given a sham manipulation to control for the handling involved

during the infection procedure. We chose these two infection intensities because they fall well within the range normally encountered in newly infected ladybirds in the field. Thus, the six treatments were: 'normal, 10', 'normal, 4', 'normal, control', 'low, 10', 'low, 4', 'low, control'. All ladybirds were then maintained individually in petri dishes. As ladybirds can be susceptible to microbial gut infections when cultured in the laboratory, we replaced the dishes housing the beetles with clean dishes every day. Thus, each ladybird was checked every day and the day of death was noted for any that died before the end of the experiment on day 40 (logistic considerations dictated the point at which the experiment ended).

Assessment of parasite fecundity and transmission

We monitored the progress of all infections every 3rd or 4th day by checking the beetles' wing-cases under a dissecting microscope. The control beetles received a sham handling at the same time. On the first check, if any of the mite larvae were found not to have 'embedded' (attachment to the wing-case followed by a distinct and rapid period of growth) then additional larvae were added to restore the complement of infective larvae to the assigned 4 or 10. Further checks were made until the termination of the experiment after 40 days. We counted the number of eggs produced by each adult mite and also recorded the number of infective larvae.

On days 20 and 30, the females were given access to a male and allowed to mate. After copulation, the males were immediately removed and checked in order to determine how many mite larvae they had acquired. In preliminary experiments we found that the vast majority of mites that transfer during copulation go on to successfully attach and reproduce. Very few of the beetles were infectious by the 'day 20' copulation and consequently negligible numbers of infective mite larvae were transmitted to a male on that day. Transmission data were therefore derived from the 'day 30' copulation (by which point all infected beetles were infectious).

Data analysis

To enable us to predict how survival probabilities varied with infection intensity and nutritional stress, we used parametric survival analysis with a logistic error distribution. Censoring took place on day 40 when the experiment was terminated. Model fitting was carried out using Anova to test whether or not the removal of individual model components, beginning with the least significant, led to a significant increase in residual deviance. Fecundity data are expressed as the number of mite eggs, or larvae, per adult mite. Transmission data are expressed both as the per adult mite and total numbers of mite larvae recorded on males following copulation on day 30. Fecundity and transmission data were analysed using Anova, with a natural log transformation where appropriate. Given that survival rates differed among treatments (below), we only analysed fecundity and transmission data for those beetles that survived for long enough to copulate on day 30. All statistical analysis was carried in R (version 1.9.1 for Mac OSX).

Results

Host survival

Host survivorship was reduced significantly by nutritional stress and also by infection with *Coccipolipus hippodamiae* (Fig. 1). The minimum adequate statistical model was obtained by combining the '4' and '10' mite treatments into an 'infected' group and by removing the interaction term (model intercept $=56.25$, $n=98$, $SE=6.725$, $p<0.0001$; coefficient for nutritional stress $=12.54$, $SE=4.320$, $p=0.0037$; coefficient for infection $=-18.92$, $SE=6.736$, $p=0.0050$). The predicted mean ages at death derived from the model (i.e. the mean ages at which beetles would be expected to die in the absence of censoring) indicate that nutritional stress reduced survival by a very similar amount in uninfected and infected beetles. The mean survivorship of the uninfected ladybirds was predicted to decrease from approximately 69 to 56 days in response to food stress, whereas the mean

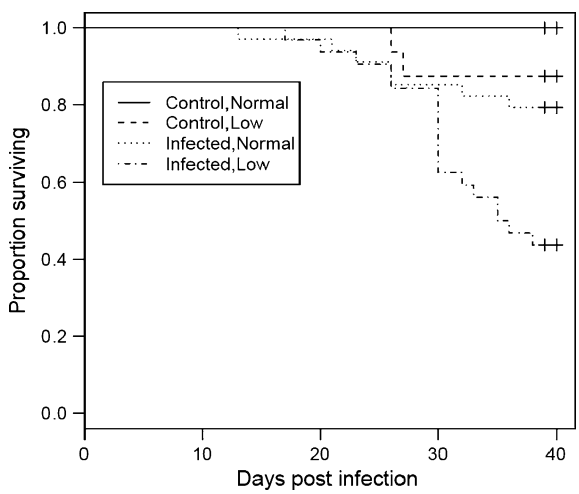

Fig. 1. Proportion of ladybirds surviving with time following initial infection or control treatment, for ladybirds given access to a normal ('Normal') or restricted ('Low') diet. See text for details.

survivorship of infected ladybirds was predicted to decrease from approximately 50 to 37 days when subjected to food stress.

Parasite fecundity and transmission

Higher infection intensity was associated with a significant reduction in mite fecundity. Thus, significantly fewer eggs were produced per adult mite on ladybirds infected with 10 mites than on ladybirds infected with 4 mites ($F_{1,53} = 32.02$, $p < 0.0001$). Fecundity was also significantly reduced when the host was subjected to nutritional stress ($F_{1,53} = 23.40$, $p < 0.0001$). There was no significant interaction between these factors and the interaction term was dropped from the final model. When the ladybirds were fed normally, increasing infection level from 4 to 10 mites decreased the mean number of eggs produced per adult mite from 25.2 to 14.3 (Fig. 2a). When the ladybirds were nutritionally stressed, the mean number of eggs per mite decreased from 15.9 to 10.1 when infection intensity increased.

The effect of infection intensity on fecundity was mirrored by a significant reduction in the number of larvae that successfully developed per adult mite ($F_{1,\ 53} = 6.09$, $p = 0.0168$). Thus, significantly fewer larvae were produced per mite on ladybirds infected with 10 mites than on those infected with 4 mites. The number of larvae produced per adult mite was also significantly reduced by host nutritional stress ($F_{1,53} = 16.08$, $p = 0.0001$). Once again, there was no significant interaction between these factors and the interaction term was dropped. On normally fed ladybirds, the mean number of larvae produced per adult mite decreased from 7.5 to 4.3 when infection level was increased (Fig. 2b). When the ladybirds were nutritionally stressed, the mean number of larvae produced per adult mite decreased from 4 to 2.6 larvae as infection intensity increased.

Host nutritional stress significantly reduced both the number of larvae transmitted per adult mite (Fig. 2c; $F_{1,52} = 32.55$, $p < 0.0001$) and the total number of larvae transmitted ($F_{1,52} = 33.41$, $p < 0.0001$). Although higher infection intensity significantly reduced mite fecundity (above), we did not detect a corresponding drop in the number of larvae transmitted per mite when ladybirds were infected with 10 rather than 4 mites, taking the data set as a whole (Fig. 2c; $F_{1,52} = 0.12$, $p = 0.74$). However, there was a significant interaction between infection intensity and host nutritional stress for per mite transmission ($F_{1,52} = 4.38$, $p = 0.041$). Further analysis showed that the effect of infection intensity on the number of mite larvae transmitted per adult mite depended on host condition (Fig. 2c): transmission per mite increased

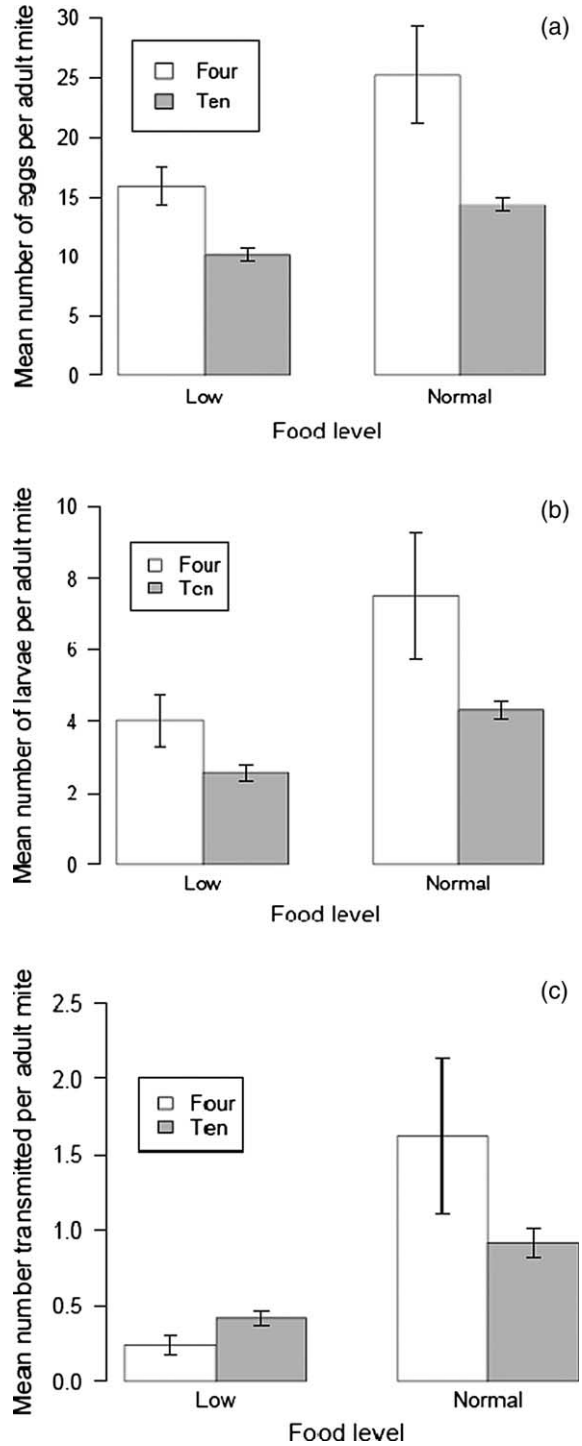

Fig. 2. Effects of infection intensity ('Four' and 'Ten') and food treatment ('Normal' and 'Low') on: (a) mean number of eggs produced per adult mite; (b) mean number of infective larvae successfully developing per adult mite; and (c) mean number of mite larvae transmitted per adult mite. Error bars show +/− one standard error for untransformed data. See text for details.

with infection intensity by a small but significant amount when the ladybirds were nutritionally stressed ($F_{1,25} = 5.31$, $p = 0.030$), but did not change significantly when diet was unrestricted ($F_{1,27} = 1.74$, $p = 0.197$). Overall, the total number of mite larvae transmitted was significantly higher when ladybirds were infected with 10 mites ($F_{1,52} = 26.61$, $p < 0.0001$).

Discussion

Parasite fecundity is an important determinant of the basic reproductive ratio of a parasite (R_0) and has an important influence on transmission dynamics (Tompkins et al. 2002). However, we know of no other experimental studies of within-host constraints on fecundity that have assessed the effect on transmission. Our results demonstrate that parasite fecundity is constrained by increased infection intensity in the *Coccipolipus hippodamiae – Adalia bipunctata* system, because mites developing on more heavily infected beetles had lower per capita rates of egg production. Mites also produced fewer eggs when their hosts' access to food was restricted. Our data show that ladybird survival was compromised when diet was restricted (even in the absence of infection), so we conclude that parasite fecundity is also constrained by host condition. These results were mirrored for the numbers of infective larvae produced per adult mite (which were also lower when infection intensity was increased and host condition was reduced).

Overall, therefore, the fecundity data suggest that infection intensity and host condition may both constrain transmission. In contrast, when the ladybirds were fed normally, infection intensity did not significantly affect the numbers of infective larvae transmitted per adult mite. Additionally, when the ladybirds were nutritionally stressed, a small but significant increase occurred with increased infection intensity, possibly indicating that mite behaviour was altered (e.g. increased motility and infectivity). Significantly more larvae were also transmitted in total from ladybirds infected with more adult mites, regardless of host nutritional status. However, significantly fewer mite larvae were transmitted (both per adult mite and total numbers) when host condition was compromised, regardless of infection status. Given the lower fecundity of the adult mites on nutritionally stressed hosts, the most parsimonious explanation for this difference is that fewer mite larvae were available to transfer to the new host. Thus, under the conditions imposed in this study, host condition appears to have placed a stronger constraint on transmission than infection intensity.

A interesting caveat to this result is that our experimental design only allowed us to examine transmission in relation to a single mating (following an earlier mating when very few beetles were infectious; see above), whereas detailed field studies have shown that *A. bipuntata* can mate every 2 or 3 days under natural conditions (Webberley et al. 2006a). It would therefore be interesting from an epidemiological perspective to determine whether or not transmission is affected over repeated matings. Given the magnitude of the fecundity effects demonstrated in the present study, we anticipate that we would observe similar constraints on transmission, although repeated matings may reveal (in particular) a more marked effect in relation to infection intensity than we were able to detect. Further experiments would be required in order to test this possibility.

Host condition or nutritional status is an understudied aspect of parasite ecology. Whilst it seems logical to assume that host nutritional stress would constrain parasite fecundity by reducing the pool of resources that is available to the parasite, it may also release parasites from immune-mediated constraints and thereby allow more reproduction (Krasnov et al. 2005). However, *A. bipunctata* appear to lack an effective immune response to *C. hippodamiae*, so it is likely that the change in fecundity arose because the mites were less able to acquire nutrients from their host's haemolymph. Recent studies in invertebrates have produced similar conclusions (Ebert et al. 2000, Bedhomme et al. 2004, Logan et al. 2005). These studies focussed on classical microparasites in hosts that lacked immunity or else used parasite doses that favoured successful host infection. We have added to this body of work by showing that transmission can also be constrained. Our data do not allow us to quantify the effect on per-contact transmission probabilities, but it is very likely that this parameter will be affected as well, particularly given that *A. bipunctata* can mate every 2 or 3 days in their natural habitat (Webberley et al. 2006a).

Higher infection intensity was associated with reduced fecundity, but our data no not demonstrate conclusively that the mechanism underlying this effect was increased competition for a limited pool of resources. It is possible (if unlikely) that infection may only be associated with a marginal resource cost to the host, such that increases in infection intensity per se have little effect on the host. Instead, constraints on fecundity may arise if mites occupying the same host are agonistic towards one another in some way, or compete for space rather than the host's nutritional resources. Either mechanism could lead to reduced fecundity at higher infection intensities. Two lines of evidence support this alternative hypothesis. First, our analysis failed to detect an effect of infection intensity per se on host survival, despite the fact that ladybirds with higher infection intensities carried over double the number of

adult mites. Second, we did not detect an interaction between the effects of host condition and infection intensity on mite fecundity – but the 'resource cost' hypothesis predicts that the effect of infection intensity on fecundity would depend on host nutritional status. Further work would be needed to test these ideas.

More generally, theoretical models of STD dynamics tend to predict that host-parasite co-existence is less likely when infected individuals cannot reproduce, particularly given that transmission is generally assumed to be frequency-dependent for STDs (Getz and Pickering 1983, Thrall et al. 1993). In one sense, this makes co-existence in the *C. hippodamiae* – *A. bipunctata* system difficult to explain. However, the dynamics of the system in the field are complex and field work demonstrates that the phenology of the beetle is likely to be stabilizing (Webberley et al. 2006a). There are several epidemic peaks of infection within each year, with prevalence often approaching 100%, and each epidemic is eventually diluted by the recruitment of new uninfected beetles from the following generation. The transmission dynamics also have a strong element of density-dependence, which may further help to stabilize dynamics (Ryder et al. 2005).

The data we present here raise further questions about the system's dynamics. With the recruitment of the first new generation of uninfected beetles in a typical summer (Webberley et al. 2006a), population density increases. The previous, over-wintered generation, in which *C. hippodamiae* prevalence will tend to be high, is effectively engulfed by the new generation and prevalence is strongly diluted. A new epidemic wave of infection then begins as the two overlapping generations inter-breed. The rate at which the epidemic grows will be much influenced by the magnitude of R_0. Significantly, in the over-wintered generation, infection intensity will tend to be very high, because each infected individual will carry adult mites from iterant rounds of reproduction. Host body condition may also be low among infected beetles, which, as well as being heavily infected, will be senescent. Therefore, in light of the results that we have presented here, we note that R_0 may vary with the strength of the fecundity and transmission effects that are associated with host condition. Further experiments would be required to quantify the effect on per-contact transmission probabilities, but we expect that these factors will partly determine the shape of the epidemic curve.

Various studies have demonstrated a survival cost of parasite infection (Brown et al. 1995, Polak 1996, Altizer and Oberhauser 1999, Jokela et al. 1999, Siva-Jothy and Plaistow 1999, Tsubaki and Hooper 2004). Our analysis further quantifies the mortality component of the virulence of *C. hippodamiae*. A previous study established that these mites rapidly induce sterility among the female ladybirds, but the same study failed to find a significant effect of infection on mortality (Hurst et al. 1995). Subsequent investigation showed that the mites do cause an increase in over-wintering mortality in the field (Webberley and Hurst 2002), but here we have shown that the mortality cost is unlikely to be confined to the over-wintering generation. In a study of a related sexually transmitted mite, *Chrysomelobia labidomerae*, which infects milkweed leaf beetles, *Labidomera clivicollis*, Abbot and Dill (2001) also found that mite infection lead to reduced survival under food stress. Although nutritional stress increased mortality in our study, the increase in mortality associated with infection was not dependent on poor nutrition. However, survival should clearly be most strongly affected in the field when infected populations experience periods of food stress (Fig. 1). We also note that reduced longevity among infected ladybirds may reduce transmission between generations, which would contribute towards the dynamics of infection within each cohort.

In conclusion, theoretical models of STD dynamics make fundamentally different predictions from most other types of host-parasite model (including, for example, the possibility of deterministic extinction), and yet empirical data on even basic model parameters are sparse. Here we have demonstrated that important within-host constraints on parasite fecundity and transmission in an animal-STD system are closely akin to those often reported for many non-sexually transmitted diseases (Ebert et al. 2000, Bedhomme et al. 2004, Logan et al. 2005). This adds to the growing picture that STDs share many epidemiological features with such diseases (O'Keefe 2005), but also underlines the need to acquire more experimental data as the number and diversity of known animal-STD systems continues to grow (Lockhart et al. 1996, Knell and Webberley 2004).

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