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Sexually-transmitted disease in a sub-tropical eucalypt beetle: infection of the fittest?

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Abstract

1. The ecology of sexually-transmitted diseases (STDs) is 0topical in scientific research, and their demography and epidemiology differ from those of classical pathogens and parasites. Transmission of STDs is generally density-independent, occurs via the "fittest" individuals in a population (or, at least, those that achieve the most matings), and reflects differential mating success. STDs can therefore have a major influence on the evolution of host mating systems.
2. We studied the epidemiology of a recently described STD of a chrysomelid beetle in applied and theoretical contexts, exploring the virulence, intensity and prevalence of infection, and using our results to test ecological predictions.
3. *Chrysophtharta cloelia* is infected with a sexually-transmitted mite (the STD), *Parobia captivus.* Infection rate over three beetle generations (seven months) was determined and the STD's effects on fertility, fecundity, longevity, mating success and overwintering survival was measured.
4. Throughout the season around 40% of beetles were infected, with approximately one quarter of such hosts carrying infective life stages of the STD at any one time. Infection by *P. captivus* significantly decreased overwintering survival, but did not impact on other fitness parameters measured, including mate acceptance. However, more female beetles were infected than male beetles, while within both sexes larger beetles were more likely to be infected.
5. Our results concur with theoretical predictions that STDs may be selected for low virulence and low detectability, and provide empirical support for hypotheses regarding variable mating success and mating skews.

Introduction

The ecological significance of sexually-transmitted diseases (STDs) has historically been overlooked (Kokko et al. 2002; Webberley & Hurst 2002), but is now receiving significant attention (e.g. Thrall et al. 2000; Welch et al. 2001; Boots & Knell 2002; Kokko et al. 2002; Moore & Wilson 2002; Webberley & Hurst 2002; Webberley et al. 2002; Knell & Webberley 2004; Webberley et al. 2004, 2005). Sexually-transmitted diseases differ demographically and epidemiologically from classical pathogens and parasites: their transmission is generally considered frequency- rather than density-dependent (Knell & Webberley 2004; but see Ryder et al. 2005), occurs via the "fittest" individuals in a population, and reflects differential mating success.

For susceptible host organisms, the trade-off between the advantages of multiple mating and the risk of acquiring STD infection defines optimal reproductive strategy, and illustrates the potential importance of parasites in the evolution of mating systems (Boots & Knell 2002; Kokko et al. 2002). STDs may influence the mating system of their hosts in two ways: indirectly, if selection pressure against mating with an infected partner is strong; or directly, if STD infection alters the behaviour of infected individuals to increase matings and therefore transmission (Webberley et al. 2002, 2004). The paradox of STD infection – whereby the "attractive" or "fitter" individuals in a population (those that attain the most matings) are more likely to acquire and transmit the STD – means that STDs can have the opposite effect on mating systems than other forms of infection. (Thrall et al. 1997, 2000). Yet, despite selection pressure being expected to favour individuals whose behaviour reduces the number of sexual encounters, and therefore the

risk of STD infection, especially where STDs are detrimental to the host, monogamy is rare in natural systems (Boots & Knell 2002). Knell (1999) predicted selection for reduced virulence in STDs as a consequence of their transmission being dependent upon host mating success. Generally, especially for male hosts, increased promiscuity equates to greater fitness (see Thornhill & Alcock 1983), but in the presence of STDs increased promiscuity also results in a higher likelihood of infection. Hosts and their STDs both, therefore, share congruent interests in facilitating host mating success, which in turn favours selection for low virulence among STDs (Knell 1999; Nunn & Altizer 2004).

In a review of sexually-transmitted diseases in animals, arthropods as STDs represented less than 2% of cases, and were recorded as hosts in less than one-fifth of cases; with regards to invertebrate STDs the authors were "…frequently frustrated by the lack of concrete data" (Lockhart et al. 1996). Furthermore, because of the short lifespan of insects and differences in voltinism of populations, the dynamics of STDs in insects are likely to differ from those of more-studied mammalian systems (Welch et al. 2001). STD persistence in populations relies on overlap in generations (e.g. Seeman & Nahrung 2004; Webberley et al. 2004, 2005), which in insects depends to some degree on seasonal conditions (e.g. Nahrung & Allen 2004a).

In Australia, the recent discovery of podapolipid mites as STDs in endemic coleopteran fauna (Seeman & Nahrung 2003, 2005) provided the opportunity to explore the prevalence, transmission, and virulence of a STD-host system in the subtropics. The Coleoptera suffer more STDs than other insect orders, while podapolipid mites represent

an important taxon of insect STDs (Knell & Webberley 2004), whose effects range from neutral (e.g. Baker & Eickwort 1975) to deleterious (e.g. Hurst et al. 1995); some have even been considered as biological control candidates (e.g. Drummond et al. 1985). Our research focuses on a recently described sexually-transmitted mite (*Parobia captivus* Seeman & Nahrung - the STD) that parasitises *Chrysophtharta cloelia* (Ståhl) (Coleoptera: Chrysomelidae), a pest of eucalypt plantations in eastern Australia. Specifically, we collected seasonal demographic field data to measure the temporal prevalence and intensity of STD infection, and conducted laboratory trials to determine the impact of STD infection on host fecundity, longevity, fertility, mate choice and overwintering survival. Host characteristics, including melanistic polymorphism and sexual size dimorphism, proved this host-parasite system an ideal model to test predictions of female bias in STD infection (Thrall et al. 2000), female-bias in immunocompetence (Rolff 2002), size-bias in parasite infection (Moore & Wilson 2002) and to test the notion that cuticular melanism is associated with resistance to parasite infection (see Robb et al. 2003).

Materials and Methods

Study system

The host in our study, *C. cloelia*, is a paropsine chrysomelid beetle of economic importance to sub-tropical hardwood forestry in eastern Australia (Simmul & de Little 1999; Carnegie 2002). Two adult colour morphs are widespread in *C. cloelia* populations: an orange-brown form and a melanic form (Selman 1994; Carnegie 2002). Beetles are infected with a parasitic podapolipid mite (the STD), *P. captivus* (Seeman &

Nahrung 2005) which, like many of the Podapolipidae (Knell & Webberley 2004), is sexually-transmitted (pers. obs.). Our field site was a 7.2 ha, 3-year-old *Eucalyptus dunnii* Maiden plantation established on ex-grazing land near Beaudesert (28°0'3.24"S 152°55'26.4"E), Queensland, Australia. Total rainfall during our sample period (September 2004 – April 2005) was 453 mL and average daily temperatures ranged between 13.5 °C and 29.8 °C (min 5 °C, max 38 °C).

Seasonal prevalence within and between hosts

Mating pairs and single adult *C. cloelia* from throughout the plantation were collected into separate plastic vials every two weeks between September 2004 and April 2005. An average of 75 ± 8 (range 23 – 115) adults were collected in each sample. Beetles were sexed in the laboratory based on tarsal differences (Baly 1862), their length from tip of clypeus to posterior elytra was measured to the nearest 0.01 mm using a digital vernier caliper, and their colour noted. Maturity was determined by compressing their elytra: deflection indicated that beetles were still teneral and therefore sexually immature (see Nahrung & Allen 2004b), and allowed non-destructive assessment of when new generation beetles entered the population. All beetles were examined for mites by raising their elytra to view the elytral undersurface, dorsum, hindwings and mesepimeron under a dissecting microscope (x40) and the number of eggs, larvae and adults was counted.

Sex-, colour-, size-based prevalence of mite infection

Using the data obtained as described above, we tested for possible differential infection prevalence between sexes using Pearson's Chi square test (specifically to ascertain whether this beetle shows female bias in infection as predicted by STD theory but rarely demonstrated in natural systems (Thrall et al. 2000; Kokko et al. 2002)). Further, we used the results of laboratory experiments (below) to investigate the possible mechanisms behind such bias. We also tested the prediction that melanism may result in greater resistance to parasites (see Robb et al. 2003) by comparing the seasonal infection rates between black and orange beetles using Pearson's Chi square test. Finally, we used our size data to compare sex, size and infection status using a 2-way ANOVA.

Mite impacts on longevity, fecundity and fertility

Beetles were collected from the field site in October and November 2004 and their sex, infection status and parasite load determined as described above. Twenty infected and uninfected females, and twenty-five infected and uninfected males were established in separate Petri dishes for longevity studies, but three males (one infected, two uninfected) escaped during the trial so were excluded. Average infection intensity of infected beetles was 21 ± s.e. 4.5 mites (all stages) per host (range 1-146). Beetles were provided moist filter paper and fresh *E. dunnii* foliage that was changed every three or four days when survival was recorded. Data were checked for differential survival between sexes using a t-test; sexes were subsequently combined and data analysed using a Kaplan-Meier

survival curve (Kaplan & Meier 1958) and non-parametric pairwise comparisons were made to determine differences between infection status ($P<0.05$).

A subsample of females was chosen without conscious bias from the longevity trial for fecundity and fertility assessment. The eggs laid by twelve infected and twelve uninfected females were collected and counted twice each week to measure fecundity. Egg batches from each female were held separately in clean Petri dishes for larval emergence. Fertility rates were calculated by dividing the number of hatched larvae by the initial number of eggs. Fecundity was compared between infected and uninfected beetles using a Kruskall-Wallis test, and hatch rates were arcsine-square root transformed, then analysed using a t-test. Pearson correlations were used to identify whether the number of mites per beetle related to fecundity and fertility.

Mite impacts on mating

Beetles collected from the field in December and January were used for laboratory choice and no-choice mating trials. No-choice tests comprised housing male-female pairs of beetles of all possible infection status combinations (i.e. infected female × uninfected male; infected female × infected male; uninfected female × uninfected male; uninfected female × infected male) in separate Petri dishes with fresh *E. dunnii* foliage. Infected beetles had all mite stages present, but parasite load was not controlled for. Twenty-five replicates of each combination were established, observed for three hours and the time taken for pairing was recorded. The time spent paired was then recorded for up to six hours; pairs that exceeded this time were recorded as 6h. Mite transfer from

combinations that allowed its assessment (i.e. infected female × uninfected male; and uninfected female × infected male) was determined by inspecting the initially uninfected partner for mites transferred during mating. Such observations were used to verify sexual transmission, and to investigate potential differences in mite dispersal between males and females that might engender sex-biased prevalence in infection.

Choice-trials were conducted using all possible combinations of male-female beetles (i.e. infected female with an uninfected and an infected male; uninfected female with an uninfected and an infected male; infected male with an uninfected and an infected female; uninfected male with an infected and an uninfected female). Twenty-five replicates of each combination were established, and observed for three hours or until a mating pair was formed. The time taken for pairing to occur was recorded, and the single beetle in such treatments was removed and checked for mites to confirm which of the test beetles was mating. Mating pairs were allowed to mate until they separated or for six hours following initial pairing, whichever occurred sooner. The time spent mating was recorded as for no-choice trials, and mite transfer from susceptible pairings was again determined by examining potential recipient hosts. Data from both trials were analysed using Pearson's chi-square tests to determine whether pairings in each combination deviated from equivalence. Time to mate and time spent mating for each trial were analysed using non-parametric Kruskall-Wallis tests ($P<0.05$) because data were not normally distributed.

Mating *C. cloelia* pairs were collected from the field between late November 2004 and January 2005. Infection status of males and females within such pairs was used to determine the frequency of mating combinations in the field. Pairs from which pre-mating infection status could not be unequivocally assigned (*i.e*. because mites may have transferred during that mating, based on the presence of dispersive stages) were discarded from analysis. Numbers of infected and uninfected males and females present in the field during this timeframe were used to calculate mating frequency of all possible infection × sex combinations if mating were random, and compared using a two-way contingency table.

Mite impacts on overwintering survival

Pre-overwintering beetles were collected from the field in late March 2005 and their infection levels (number of mites per beetle) were recorded. Twenty infected and twenty uninfected beetles (ten of each sex) were placed individually in Petri dishes and provided fresh *E. dunnii* foliage which was changed weekly when mortality was recorded and Petri dishes were cleaned. Infection intensity of infected beetles averaged 24 ± s.e. 3.1 mites (all stages) per host (range 3 – 49). Beetles were deemed to be overwintering when feeding and defecation ceased for a minimum of 14 consecutive days (see Nahrung & Allen 2004b). Data were checked for differential survival between sexes using a t-test; sexes were subsequently combined and data were analysed using a Kaplan-Meier survival curve (Kaplan & Meier 1958) and non-parametric pairwise comparisons were made to determine differences between infection states ($P<0.05$). A Pearson correlation was

employed to ascertain whether there was a statistical relationship between overwintering survival duration and initial mite infection level.

Results

Seasonal prevalence of infection

Throughout the active field season *P. captivus* infected 38.3 ± s.e. 3.6 % (17.4 – 69.2 %, total n = 1121) of sexually-mature *C. cloelia* (Figure 1). The infection rate at the end of the season (pre-overwintering) was significantly higher than the infection rate at the beginning of the season (post-overwintering) (Pearson chi-square test, $\chi^2_1 = 15.7$, $P < 0.001$). The spread of mites throughout the population increased approximately linearly ($y = 0.064x + 0.065$, $R^2 = 0.82$, $P = 0.006$) until the appearance in the field of uninfected new generation beetles in summer. During the overlap in generations the spread of mites showed no linear pattern ($R^2 = 0.09$, $P = 0.7$), but linearity resumed within the new generation once new beetles ceased entering the population in February ($y = 0.057x + 0.234$, $R^2 = 0.81$, $P = 0.04$). No field-collected teneral beetles were infected with mites (n = 57).

Figure 1: Proportion of sexually-mature *Chrysophtharta cloelia* (O), and proportion of such beetles infected with mites (△) in the field throughout their active season. The period between 29 December 2004 and 9 February 2005 (circled) illustrates the effect of new-generation beetles on mite prevalence.

Infection of sexes and colour morphs

Whole-season infection rates showed a female bias, with significantly more female beetles infected with mites than males (45.4 and 36%, respectively) ($\chi^2_1 = 10.12$, $P = 0.001$). This significance arose following the emergence of new generation beetles into the population (Figure 2); overall, parental generation beetles lacked sex bias ($\chi^2_1 = 1.35$, $P = 0.25$), whereas subsequent generations exhibited a strong female bias ($\chi^2_1 = 7.0$, P =

0.008). Black beetles represented 15% of the population, regardless of sex ($\chi^2_1 = 0.007$, P = 0.93), and both colour forms were equally infected with mites ($\chi^2_1 = 0.024$, P = 0.88).

Figure 2: Prevalence of mite infection for male (o) and female (△) *Chrysophtharta cloelia* by *Parobia captivus* throughout the field season at Beaudesert, Queensland.

Field-collected female beetles were significantly larger than males (2-way ANOVA, sex: $F_{1,1116} = 753.4$, P < 0.001), while within sexes, infected beetles were significantly larger than uninfected beetles (infection status: $F_{1,1116} = 10.17$, P = 0.001); there was no interaction between sex and infection status with respect to size (sex*infection status: $F_{1,1116} = 0.017$, P = 0.89) (Table 1), nor a correlation between beetle size and intensity of infection (number of mites/beetle) (Pearson correlation: Bartlett chi-square statistic = 0.314, df = 1, P = 0.58). Beetle size did not differ according to colour (t-test, $t_{1118} = 0.8$, P = 0.4).

Seasonal intensity of infection

Total mite numbers (all stages) per infected beetle differed significantly throughout the season (2-way ANOVA, $F_{12, 373}= 7.6$. P < 0.001) (Figure 3), but remained similar on males and females ($F_{1, 373} = 2.8$, P = 0.09), with no seasonal interaction according to sex ($F_{12,373} = 0.614$, P = 0.83). The highest number of mites of all stages recorded on a single beetle was 146, while the seasonal infection intensity averaged 17.1 ± 0.9. Throughout the season, an average of 26.7 % of infected beetles bore dispersive mite life stages (Figure 3).

Figure 3: Mean + s.e. number of *Parobia captivus* mites (all life stages) infecting *Chrysophtharta cloelia* throughout the field season (columns), and the proportion of infected beetles with dispersive life stages present (line).

Mite impacts on fecundity, fertility and longevity

Longevity did not differ between male and female beetles (t-test, t_{87}= 0.49, P = 0.62) so survival data were pooled for subsequent analyses. Kaplan-Meier survivorship curves (Figure 4) and their pairwise comparison (T = 1.13, P = 0.29) showed that longevity was unaffected by mite infection, while Pearson correlation showed no relationship between number of mites and longevity (χ^2_1= 0.37, P = 0.54).

Figure 4: Survivorship curve for *Chrysophtharta cloelia* adults infected (grey) and uninfected (black) with *Parobia captivus.*

Likewise, mite infection did not affect female fecundity (Kruskall-Wallis test, H_1 = 78.5, P = 0.71) or egg hatch rate (t-test, t_{22}=1.69, P=0.11) (Table 2), nor was there a significant relationship between intensity of infection (number of mites per beetle) and fecundity or hatch rate (Pearson correlation, P = 0.19, P = 0.29, respectively).

Mite impacts on mating

Overall, mating occurred in only one-third of replicates in each trial (no-choice trials 31%; choice trials 34%). There appeared to be a trend towards more mating in the infected female × uninfected male mating combination (Figure 5), but frequencies were not significantly different in any trials. Beetles did not discriminate between infected and uninfected partners in no-choice (Pearson chi-square test, χ^2_3= 2.76, P = 0.43) and choice (Pearson chi-square test, χ^2_3= 5.17, P = 0.16) trials; nor did field mating combinations

differ from those expected by random pairing ($\chi^2_3 = 4.67$, P = 0.19) (Figure 5). There is thus no evidence for STD infection altering libido or choosiness of hosts. The size of males (t-test, $t_{202}=1.8$, P = 0.07) and females (t-test, $t_{193}=1.3$, P = 0.19) did not differ between those collected whilst mating and those collected singly from the field. There was likewise no difference in the frequency of larger beetles as successful mating partners in mate choice tests over the smaller beetle in each pair (chi-square test, $\chi^2_1 = 2.3$, P = 0.13).

Figure 5: Frequency of mating combinations between *Chrysophtharta cloelia* females (F) and males (M) infected (i) and uninfected (u) by *Parobia captivus* in laboratory no-choice (black) and choice (grey) trials, collected as mating pairs from the field (white), and expected random combinations (striped) based on field prevalence.

Time to initiate mating did not differ between mating partner combinations in no-choice (Kruskall-Wallis test, $H_3= 3.25$, P = 0.36) or choice (Kruskall-Wallis test, $H_3 = 3.05$, P = 0.38) trials (Table 3). Most beetle pairs mated for the maximum trial duration (median time spent mating was 360 minutes (range 5 – 360)), a pattern that did not change according to mate-infection combinations (no-choice: $H_3= 4.05$, P = 0.26; choice: $H_3= 4.8$, P = 0.18).

Mite transfer occurred between infected and uninfected beetles in all infected-uninfected mating combinations. Larval female mites moved from infected females to uninfected males in 43% of matings, and from infected males to uninfected females in 36% of matings; these frequencies were not statistically different (Chi-square test, $\chi^2 = 0.18$, P = 0.63).

Mite impacts on overwintering survival

Overwintering survival was similar between males and females (t-test, $t_{38} = 0.69$, P = 0.42), so sexes were pooled for analyses. Mite infection significantly reduced overwintering survival (T = 96.4, P < 0.001), with no infected beetles surviving in the laboratory longer than 35 days (Figure 6). In contrast, 20% of uninfected beetles entered diapause (not feeding or producing frass) and survived for over four months.

Figure 6: Overwintering survivorship curve for *Chrysophtharta cloelia* adults infected (grey) and uninfected (black) with *Parobia captivus.*

Within infected beetles, there was no relationship between infection intensity (number of mites per beetle) and overwintering survival time (Pearson correlation, $\chi^2_1 = 1.4$, P = 0.23).

Discussion

Peak prevalence of STD infection in insect populations averages 46% (Knell & Webberley 2004): prevalence peaked in *C. cloelia* at almost 70%. As supposed for temperate counterparts, such maximum prevalence probably reflects a time delay in recruitment of new, susceptible adult generation beetles into the population (Knell & Webberley 2004; Webberley et al. 2005): the emergence of new adults into the *C. cloelia* population disrupted the linear pattern of infection observed up to that point (Figure 1). Hence, as well as virulence levels and transmission rates, the stability of insect-STD systems relies on the timing of influxes of new susceptible hosts to the system (Webberley et al. 2005). Additionally, a sex-bias in infection prevalence appeared with

the influx of new *C. cloelia* adults into the population not exhibited by the parental generation (Figure 2).

Chrysophtharta cloelia females had a significantly higher prevalence of STD infection than did males. We consider below a number of possible explanations for this sex-based bias in infection, including: first, females are more tolerant to infection than males; second, females experience less variable mating rates than do males; third, uninfected males are more choosy than infected males; fourth, transmission of mites occurs more readily from male to female than from female to male; fifth, the larger size of females makes them more suitable as hosts, either through increased area of mite attachment points or some other intrinsic factor.

Higher female infection rates may arise through differential immunity between males and females (sensu Rolff 2002), if females are more tolerant to infection than males. Rolff's (2002) hypothesis that females should invest more in immunity than males (based on Bateman's principle that males gain fitness through increased mating rates whilst females increase fitness by increasing longevity), is not supported by our laboratory data which showed no difference in survival between males and females, nor between infected and uninfected beetles. Further, field collection data would be expected to show a female bias in sex ratio if females were longer lived, males more susceptible to effects of parasitism, or both, and this was not the case here either. However, Bateman's (1948) observation that variability in male mating rates was much higher than variability in female mating rates may explain the bias in female infection. Such a pattern is predicted

in the ecological STD models of Thrall et al. (2000) and Kokko et al. (2002), and Seeman and Nahrung (2004) proposed this as the most likely explanation for female-bias in infection of *C. agricola* populations.

Transmission of mites between sexes occurred equally in our laboratory mating experiments, and is therefore unlikely to explain the female bias in parasitism, although mites appeared to disperse more readily to females than males in Baker & Eickwort's (1975) trials with *C. labidomerae* on *Leptinotarsa clivicollis*. Mite transmission occurs equivalently between sexes in *Coccipolipus hippodamieae* on ladybird hosts (Hurst et al. 1995; Webberley et al. 2004, 2005), although infection prevalence between sexes is not reported to differ in these species.

In sexually size dimorphic mammals, the larger sex (usually males) generally exhibits a bias in parasitism prevalence (Moore & Wilson 2002), and this is also the case in *C. agricola* (Seeman & Nahrung 2004) and *C. cloelia* (this study), with the larger sex (females) exhibiting a significantly higher parasitism rate than males. However, sexual-size dimorphism is often associated with a bias in mortality, which we did not find in our experiments. Moreover, even within sexes, larger beetles were more likely to be infected. The lack of correlation between infection intensity (number of mites per host) and host size, however, suggests that this pattern is not derived through the provision of more mite attachment sites by larger hosts. Larger size in insects is often associated with greater mating success (e.g. Harari et al. 1999) and increased mating rate equates to a greater risk of acquiring STD infection. Thus, theory predicts that higher infestation rates in larger

male and female *C. cloelia* in this study may be due to differential mating success: our laboratory and field trial data do not, however, support this prediction. Given this situation we can only hypothesise that our current data do not accurately reflect mate choice in this species, or that an alternative, as yet unconsidered explanation is required to explain the observed patterns in infection prevalence.

The lack of discrimination demonstrated in our mate choice experiments, and those of Abbott & Dill (2001), Webberley et al. (2002), Nahrung & Allen (2004), and Luong & Kaya (2005) for uninfected mating partners, supports Knell's (1999) predictions that STDs are under strong selection to prevent avoidance (and thereby prevent transmission) by potential mates of their hosts. Hence, STDs are predicted to evolve increased crypticity through reduced virulence (Knell 1999), a notion also borne out by our results: infection by *P. captivus* did not affect longevity, fertility or fecundity of *C. cloelia.* Similar non-pathenogenicity is imposed on *L. clivicollis* by its STD (Baker & Eickwort 1975; Eickwort & Eickwort 1986), although the majority of insect STDs are deleterious to their hosts, most commonly by reducing fertility (Knell & Webberley 2004). Contrary to theoretical predictions, some STDs have high virulence coupled with low crypticity for mate discrimination (e.g. Webberley et al. 2004).

In addition to not affecting mate choice of uninfected beetles, infection by *P. captivus* did not influence the libido of infected beetles to engage in behaviours that may increase its transmission. Time to initiate mating and time spent *in copula* was equivalent for beetles

regardless of their infection status. No discernable differences occurred in mobility or search rate between infected and uninfected beetles in mating arenas (pers. obs.).

STD infection in *C. cloelia* significantly affected overwintering survival as demonstrated in the laboratory, and implied from the significant reduction in field prevalence between the start and end of the season. *Chrysophtharta cloelia* can undergo up to five generations in a year (Elliott et al. 1998); it is thus only the final, pre-overwintering generation in a season directly under threat from adverse affects of STD infection. The selection pressure imposed through such mortality has apparently not been strong enough to enable beetles to discriminate uninfected mating partners to ameliorate the risk of infection. Although our laboratory data suggest that the STD would go extinct from populations during winter, we assume that the additional stress imposed under laboratory conditions, and our low sample size, contributed to recording excess mortality not experienced in the field. Abbott & Dill (2001) found that STD infection significantly decreased survival of nutritionally stressed beetles, and Webberley & Hurst (2002) also reported increased mortality of infected overwintering ladybirds, especially males. In contrast, overwintering survival in the Colorado potato beetle was unaffected by mite infection (Drummond et al. 1992).

We conclude by returning to the original predictions we set out to test. With respect to the *C. cloelia* / *P. captivus* system: a predicted female bias in STD infection was recorded in field data, possibly a result of differential mating rates between the sexes; female-bias in immunocompetence was not demonstrated with respect to beetle longevity, but some

traits for which we have female data we do not have corresponding male data (eg fertility) and thus we can not completely rule out a female bias in immunocompetence; a size-bias in parasite infection was demonstrated, with larger beetles more likely to be infected, but again a clear underlying mechanism to explain the pattern is unavailable; and, finally, cuticular melanism does not appear to be associated with resistance to parasite infection. Additionally, our system supports a fundamental prediction that STDs should evolve towards low virulence and increased crypticity, with measurable pathogenicity expressed against only one generation per year.

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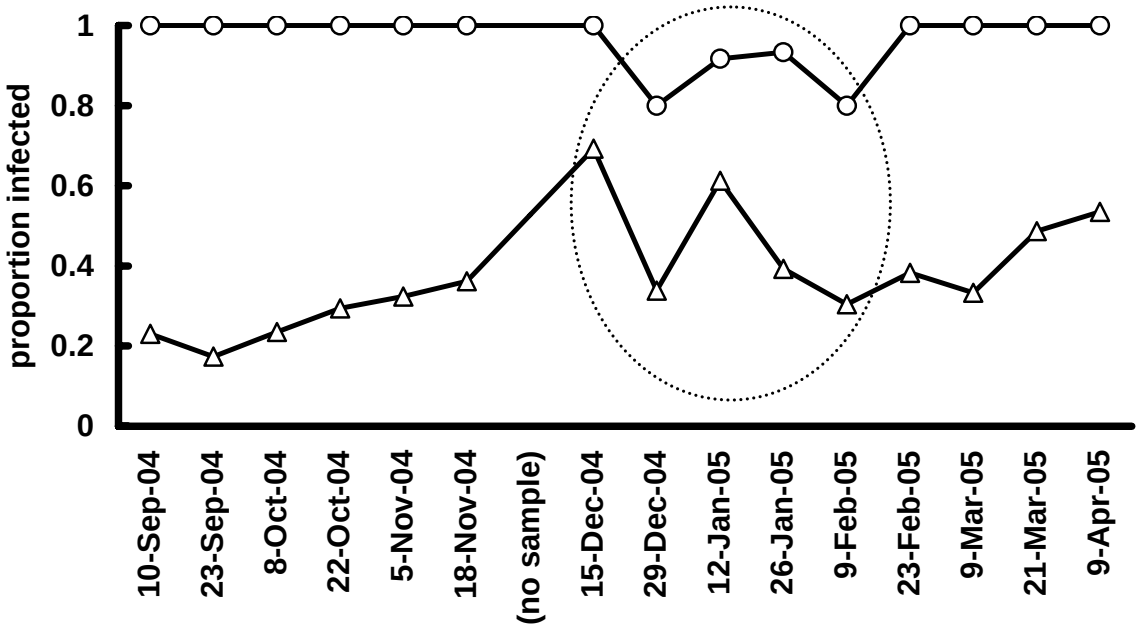

Figure 1: Proportion of sexually-mature *Chrysophtharta cloelia* (O), and proportion of such beetles infected with mites (△) in the field throughout their active season. The period between 29 December 2004 and 9 February 2005 (circled) illustrates the effect of new-generation beetles on mite prevalence.

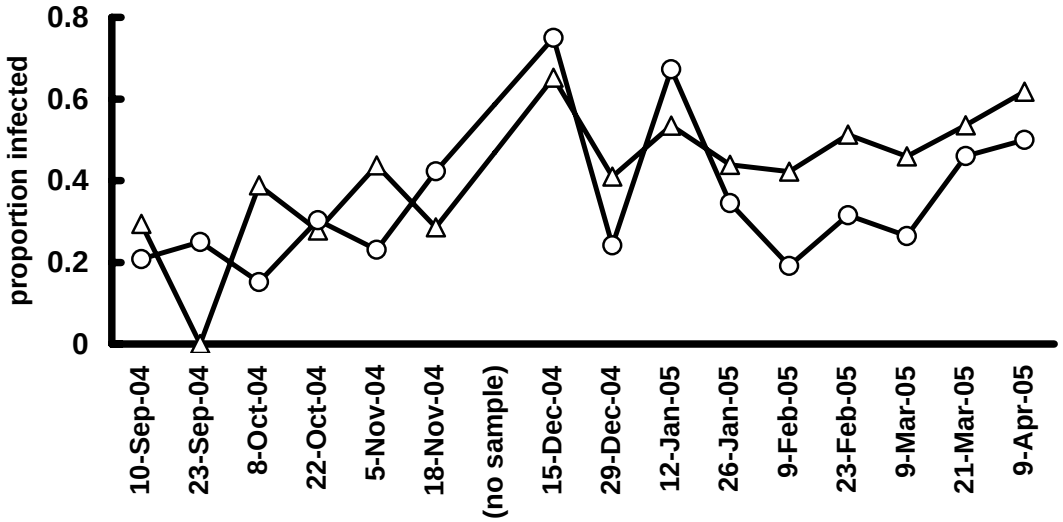

Figure 2: Prevalence of mite infection for male (o) and female (△) *Chrysophtharta cloelia* by *Parobia captivus* throughout the field season at Beaudesert, Queensland.

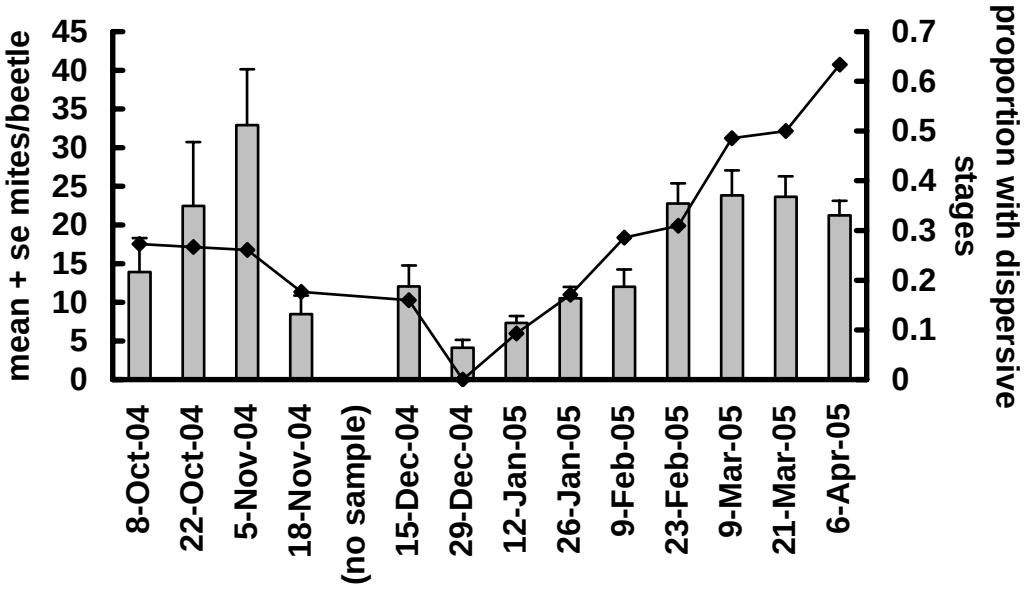

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Figure 3: Mean + s.e. number of *Parobia captivus* mites (all life stages) infecting *Chrysophtharta cloelia* throughout the field season (columns), and the proportion of infected beetles with dispersive life stages present (line).

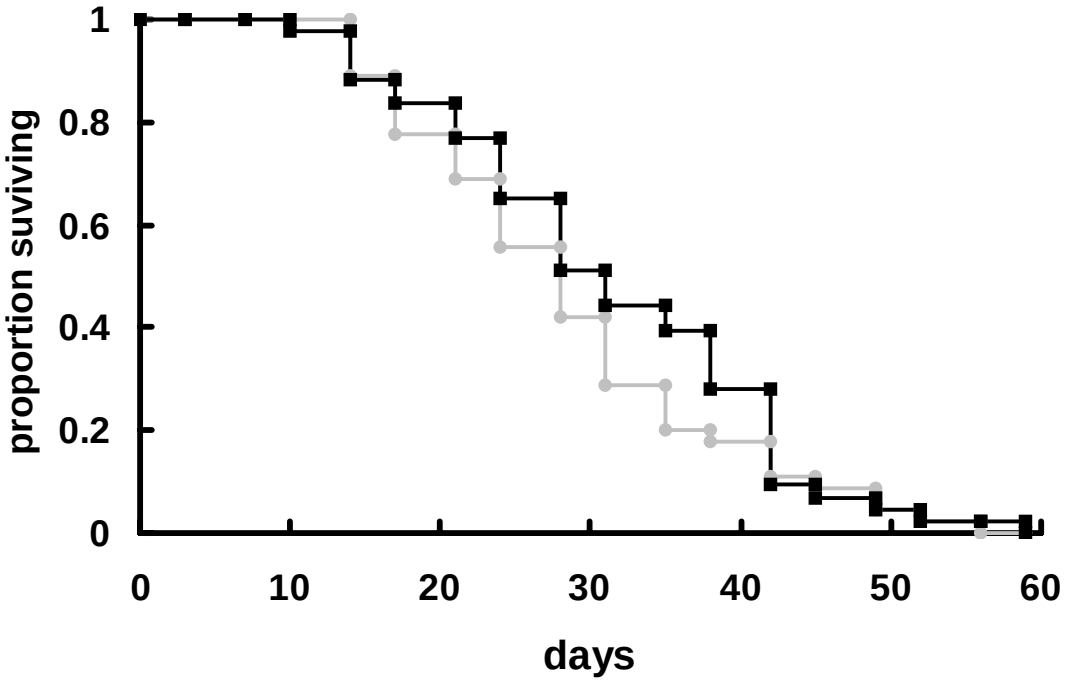

Figure 4: Survivorship curve for *Chrysophtharta cloelia* adults infected (grey) and uninfected (black) with *Parobia captivus.*

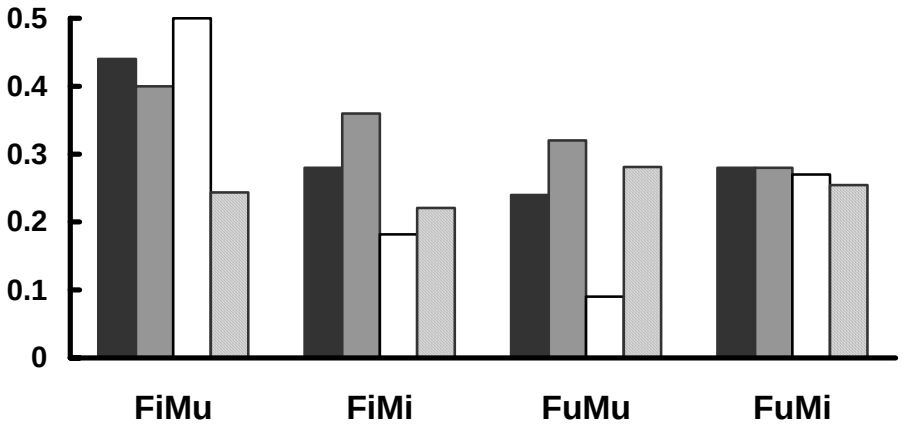

Figure 5: Frequency of mating combinations between *Chrysophtharta cloelia* females (F) and males (M) infected (i) and uninfected (u) by *Parobia captivus* in laboratory no-choice (black) and choice (grey) trials, collected as mating pairs from the field (white), and expected random combinations (striped) based on field infection prevalence.

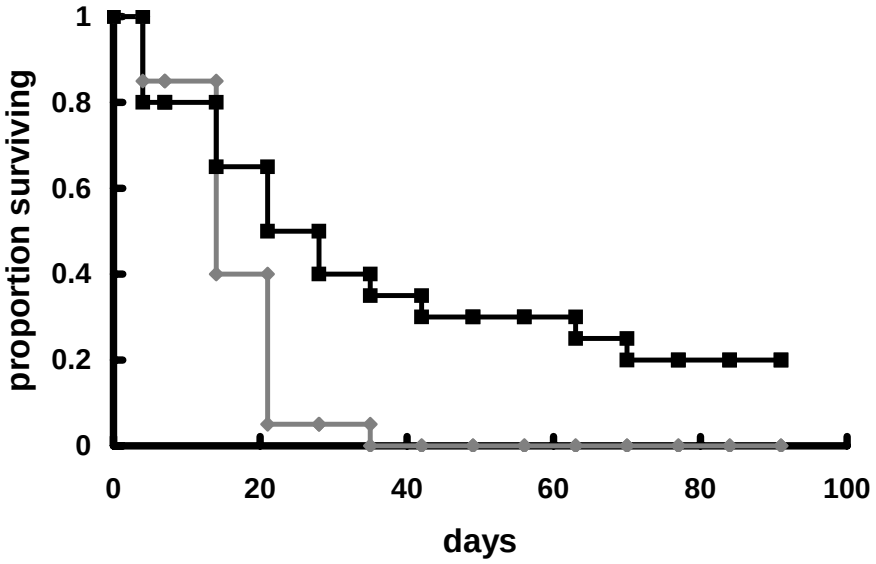

Figure 6: Overwintering survivorship curve for *Chrysophtharta cloelia* adults infected (grey) and uninfected (black) with *Parobia captivus.*

Table 1: Average ± s.e. length (range) and numbers of field-collected male and female *Chrysophtharta cloelia* infected and uninfected by *Parobia captivus.* All means differ ($P<0.05$; see text).

Chrysophtharta cloelia	size (mm)
uninfected females	7.97 ± 0.03 (6.55 – 8.96) n = 250 a
infected females	8.06 ± 0.03 (6.76 – 9.12) n = 208 b
uninfected males	7.25 ± 0.02 (5.86 – 8.39) n = 424 c
infected males	7.33 ± 0.03 (6.35 – 8.25) n = 238 d

Table 2: Mean ± s.e. (range) fecundity and hatch rate of *Chrysophtharta cloelia* females infected and uninfected by *Parobia captivus.* Means do not differ within columns (see text).

Female infection status	fecundity	hatch rate (%)
Uninfected	54 ± 16.1 (0 – 155)	98.4 ± 0.01 (93.1 – 100)
Infected	49.5 ± 7.8 (18 – 92)	99.3 ± 0.01 (94.5 – 100)

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Table 3: Time (median (range) minutes) to initiate mating in choice and no-choice mating trials between infected (i) and uninfected (u) males (M) and females (F).

	no-choice	choice
FiMu	46 (1 – 101)	124 (16 – 176)
FiMi	82 (10 – 147)	133 (14 – 180)
FuMu	26 (5 – 177)	76 (2 – 170)
FuMi	70 (1 – 168)	80 (3 – 117)

Figure 1: Proportion (O) and mite infection rate (△) of sexually-mature *Chrysophtharta cloelia* in the field throughout their active season. The period between 29 December 2004 and 9 February 2005 (circled) illustrates the effect of new-generation beetles on mite prevalence.

Figure 2: Prevalence of mite infection for male (o) and female (△) *Chrysophtharta cloelia* by *Parobia captivus* throughout the field season at Beaudesert, Queensland.

Figure 3: Mean + s.e. number of *Parobia captivus* mites (all life stages) infecting *Chrysophtharta cloelia* throughout the field season (columns), and the proportion of infected beetles with dispersive life stages present (line).

Figure 4: Survivorship curve for *Chrysophtharta cloelia* adults infected (grey) and uninfected (black) with *Parobia captivus.*

Figure 5: Frequency of mating combinations between *Chrysophtharta cloelia* females (F) and males (M) infected (i) and uninfected (u) by *Parobia captivus* in laboratory no-choice (black) and choice (grey) trials, collected as mating pairs from the field (white), and expected random combinations (striped) based on field infection prevalence.

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