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Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*

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Abstract. 1. Sexually transmitted diseases (STDs) have rarely been reported in insects and other invertebrates. The majority of those reported involve organisms where sexual transmission is augmented by either vertical (i.e. inherited) transmission, or horizontal transmission, independent of host sexual activity.

2. We here demonstrate the existence of an STD in the coccinellid beetle *Adalia bipunctata*. This species bears a parasitic mite of the genus *Coccipolipus*. We show that, like many other podapolipid mites, this mite is transmitted between host individuals at a high rate during copulation. It also appears to be transmitted at a low rate between non-copulating individuals.

3. We show that infected female *A.bipunctata* produced eggs at a reduced rate, and that the eggs produced by infected females have highly decreased viability. However, no effect of infection upon host longevity was observed.

4. The results are discussed in relation to the incidences of sexually transmitted disease in invertebrates in general, the causes of disease symptoms, and the importance of this disease in the evolution of *A.bipunctata*.

Key words. STD, venereal disease, sexually transmitted disease, *Coccipolipus*, *Adalia bipunctata*, Coccinellidae, inherited parasites.

Introduction

Sexually transmitted diseases (STDs) are known to have a considerable demographic impact on humans and other promiscuous birds and mammals (Anderson & May, 1991; Smith & Dobson, 1992; Sheldon, 1993). However, the number of well-documented incidences in invertebrates is remarkably small (Smith & Dobson, 1992). One definite example is in the Dungeness crab, *Cancer magister* (Wickham *et al.*, 1984). In this case a nemertean worm, *Carcinonemertes errans*, consumes the eggs of the female host, before producing a planktonic larval stage (Wickham, 1980). Larval nemerteans alighting upon a male individual (which produce no eggs to consume) transfer to the female during copulation. Two cases are documented in insects. In the fall army-worm, *Spodoptera frugiperda*, an ectoparasitic nematode transfers between partners during mating, and causes a discernable pathology in its host (Marti *et al.*, 1990; Simmons & Rogers, 1990). In the Indian rhinoceros beetle, *Orcytes rhinoceros*, a baculovirus is transmitted between partners, and reduces host longevity (of both male and female) and progenic survivorship (of the female) (Zelazny, 1972, 1973a, b, 1976). The status of other examples of STDs in insects has not yet been fully established. Although many insects have been suspected of bearing sexually transmitted viruses, bacteria and protozoa which produce diseases, in no case is there good evidence both that this sexual transmission occurs in the wild, and that the organism produces a fitness loss to its host. For instance, one of the most frequently cited cases of venereal transfer is that of LaCrosse virus from male to female *Aedes albopictus* (Thompson & Beaty, 1977); however, experiments have failed to show any effect on the fitness of venereally infected individuals (Patrican & DeFoliart, 1985; Patrican *et al.*, 1985, but see Grimstad *et al.*, 1980).

One other group of parasites which has been proposed as being the aetiologic agent of an STD are the mites of the

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family Podapolipidae. Podapolipid mites are found on Coleoptera, Orthoptera, Hymenoptera and Dictyoptera. They are characterized by having sessile adults which feed on the adult host, and motile larvae. These larvae have been either directly observed moving between hosts during copulation (*Podapolipoides grassi* on the locust *Chortoicetes terminifera*: Gauchat, 1972; *Coccipolipus epilachnae* on the coccinellid beetle *Epilachna varivestris*: Schroder, 1981; *Eutarsopolipus regenfussi* on the carabid beetle *Chlaenius pennsylvanicus*: Husband & Swihart, 1986), or such movement has been inferred from their position on the organism (*Ovacarus clivinae* and *O.peelei* are found attached to the vaginal membrane of their hosts, the beetles *Clivinia impressifrans* and *Pasimachus elongatus* respectively: Stannard & Vaishampayan, 1971; Husband, 1974).

The podapolipid mites certainly appear parasitic. However, studies have not explicitly demonstrated a deleterious effect on their host. In experiments where realistic numbers of mites have been placed on hosts under controlled conditions, no cost to the host has been demonstrated (Cantwell *et al.*, 1985; Hochmuth *et al.*, 1987), and observations suggest that, in other cases, the effect of mites on host fitness may be minor (e.g. Husband & Sinha, 1970). We here investigate the biology of the podapolipid mite *Coccipolipus hippodamiae* McDaniel & Morill on the coccinellid beetle *Adalia bipunctata*. *Coccipolipus hippodamiae* is an ectoparasite of adult ladybirds, engorged females being found on the underside of the elytra, towards the anterior, with larval females being found on the underside of the elytra, towards the posterior tip. We show that this organism is a sexually transmitted parasite of the coccinellid beetle *A.bipunctata* and discuss the significance of this finding.

Materials

The ladybirds used in subsequent experiments were either laboratory-reared individuals, derived either from wild-collected Moscow or Cambridge stocks. The mites used in the experiments were obtained from adult ladybirds collected in the wild in Moscow, Russia, during September 1993. The 'infected' ladybirds used bore fully engorged adult female mites (sessile) which were producing eggs. Some of these eggs had hatched: 'infected' females thus also bore larval mites.

Is *Coccipolipus hippodamiae* transmitted during copulation?

Method

Male and female ladybirds were placed in separate dishes and fed on pea aphids (*Acyrthosiphum pisum*) for at least 2 days. Four uninfected individuals of one sex (henceforth the recipient sex) were put in a large box (17 × 11 × 6 cm) with two infected and two uninfected individuals of the opposite sex (henceforth the donor sex). All ladybirds were marked with a spot of coloured Tippex^{TM} on their elytra in order to distinguish individuals. The ladybirds were observed for 1 h under a bright fluorescent light (22 W), and all contact between individuals was recorded.

After the hour had elapsed, donor sex individuals which were not mating were removed, and kept singly in petri dishes. Other individuals of the donor sex were removed following termination of mating. This prevented unrecorded matings occurring after the end of the observation period. When all donor sex individuals had been removed, the recipient sex individuals were removed to individual petri dishes.

The number of larval mites found on each of the ladybirds was recorded for each ladybird in the experiment after copulation. The experiment was performed ten times with the male as the donor sex, and ten times with the female as the donor sex. Experiments where no mating pairs formed were repeated.

Results

More mites transferred to individuals that had mated with an infected partner than to those that did not (Table 1). The null hypothesis that individuals that had mated with an infected partner received the same number of mites as individuals mated with an uninfected partner was rejected (Wilcoxon signed rank tests: for male to female transfer, $n = 6$, $T = 0$, $P < 0.05$; for female to male transfer, $n = 0$; $T = 0$; $P < 0.005$). Additionally, the null hypothesis that individuals that had mated with an infected partner received the same number of mites as individuals which did not mate was rejected (Wilcoxon signed rank tests: for male to female transfer, $n = 8$, $T = 2$, $P < 0.025$; for female to male transfer, $n = 8$; $T = 0$; $P < 0.01$). This analysis uses the mean number of mites transferred to a particular class of recipient individual where more than one recipient individual occurred in that class.

Does *Coccipolipus hippodamiae* have a cost to female hosts?

Method

Adalia bipunctata from the Cambridge population were reared in groups of five in the laboratory from laboratory-reared parents. Nine pairs of females from within these groups were isolated following emergence as adults. 15 days after emergence, four larval female mites were transferred to one member of each pair of ladybirds. The ladybirds were initially anaesthetized using a stream of carbon dioxide gas, then fixed onto their backs using a small amount of Blu-tac^{TM}. The underside of one elytron of each ladybird was then exposed, using entomological pins (size D2). The mite larvae were transferred from an infected ladybird to each experimental ladybird on the tip of an entomological pin (size E2). Two larvae were placed on each elytron. The individual from each

Table 1. The number of mites transferred to the individuals of the recipient sex, either mating with partners of different status with respect to infection, or failing to mate, in the experiment described in the text. Dashes indicate no interaction occurred.

(i) Transmission to males in the presence of two infected and two uninfected females

Experiment	Mean no. of mites transferred to males which mated with an infected female	Mean no. of mites transferred to males which mated with an uninfected female	Mean no. of mites transferred to males which did not mate during the experiment
C	13	0	0
D	5.5	0	0
G	68	0	0
H	14	1	2
J	18	0.5	–
K	–	–	0
O	13	0	0
P	29	0	0
Q	42	1	0
T	6.5	0.5	–
U	81	1.5	0

(ii) Transmissiön to females in the presence of two infected and two uninfected males

Experiment	Mean no. of mites transferred to females which mated with an infected male	Mean no. of mites transferred to females which mated with an uninfected male	Mean no. of mites transferred to females which did not mate during the experiment
A	–	0	0
B	17	0	0
E	7	–	1.5
F	4	0	0
I	23	1	1.5
L	6.5	1	0
M	13	0	18
N	16.5	–	0
R	16.5	0	–
S	12	–	1

pair which was to serve as the uninfected control was simultaneously subjected to exactly the same conditions and manipulation as the infected individual, save for the physical transfer of mites. Each individual was then placed in a separate petri dish, to prevent any later transfer of mites.

The females were thenceforth maintained on a feeding regime of seven large *A.pisum* every 2 days, alternating daily provision between four and three aphids. This level of aphids provides enough for one small clutch of eggs to be laid daily. The mites were given 7 days to establish on the female ladybirds, and then both members of the pair were mated. Males used in matings to a given pair were brothers, unrelated to the females, and free from mite infection. Following mating, all ladybirds were provided with a clean petri-dish every day to control for the stimulation to oviposition produced by changing dishes (Majerus *et al.*, 1989). The number of eggs laid on each day was recorded, and egg clutches were allowed to hatch naturally. The hatch-rate of these eggs was recorded. Females were remated every seventh day to prevent infertility arising from sperm depletion (Majerus, 1994a), members of each pair again being crossed to unrelated, uninfected, sibling males. The dishes were placed individually on the bench to prevent the differences in fecundity that may arise from to the effects of light when dishes are stacked.

The effect of mites on fecundity and egg fertility was measured over a 25-day period. At the end of this period, the number of mites on each individual was scored. Thereafter, each member of the pair was fed excess aphids for 7 days, and the viability of eggs recorded. After this time, the individuals were placed back upon the alternating three then four aphid per day diet until death, and the viability of eggs recorded. The longevity of individuals was recorded.

Table 2. The effect of mite infection on the rate of egg laying, the fertility of eggs laid, and the longevity of female *A. bipunctata*

	Infected with mites			Not infected with mites		
Pair	Eggs laid in 25 days	Proportion of eggs hatching	Lifespan of adult female (days)	Eggs laid in 25 days	Proportion of eggs hatching	Lifespan of adult female (days)
2	213	0.52	74	364	0.93	68
3	294	0.20	92	285	0.76	68
4	211	0.24	85	285	0.95	119
5	136	0.29	92	345	0.68	66
6	245	0.08	142	233	0.86	83
7	151	0.44	60	293	0.56	103
8	141	0.45	74	267	0.84	118
9	282	0.14	77	254	0.81	90
10	277	0.00	77	278	0.89	123

Results

The fecundity of infected individuals was significantly lower than that of their controls (Table 2) (paired *t*-test: $t = 2.52$; 8 d.f.; $P < 0.05$ two-tail). Egg viability was significantly lower in infected than control individuals (Table 2) (sign test: infected individual produced eggs of lower viability in all of nine cases: $P < 0.005$). No significant difference in longevity could be discerned (Table 2) (mean longevity of infected individuals = 85.9 days, SE = 7.75; mean longevity of uninfected individuals = 93.1 days, SE = 7.81; paired *t*-test; $t = 0.571$; 8 d.f.; $P > 0.5$).

The viability of eggs laid by females infected with the mite decreased markedly with time. At first, eggs laid by infected individuals had a good level of viability, but this declined, frequently to zero, within 7–9 days of the start of the experiment (Fig. 1). The eggs laid by mite-infected ladybirds after this period were a normal opaque yellow when first laid, but shrivelled and became translucent within 24 h. Normal eggs remain opaque and maintain their shape until hatching. The inviability of eggs from infected individuals was maintained even during the period of provision with excess aphids.

Between two and twelve engorged, egg producing, adult mites were found on the infected individuals after the 25 days of the fecundity test. Between 25 and 200 eggs could be discerned, and in all cases larval mites were present.

The effect of mites on the fertility of females from the Moscow population

Method

Seven laboratory reared adult female ladybirds derived from the Moscow population were each infected with six larval mites as described previously, and the females fed

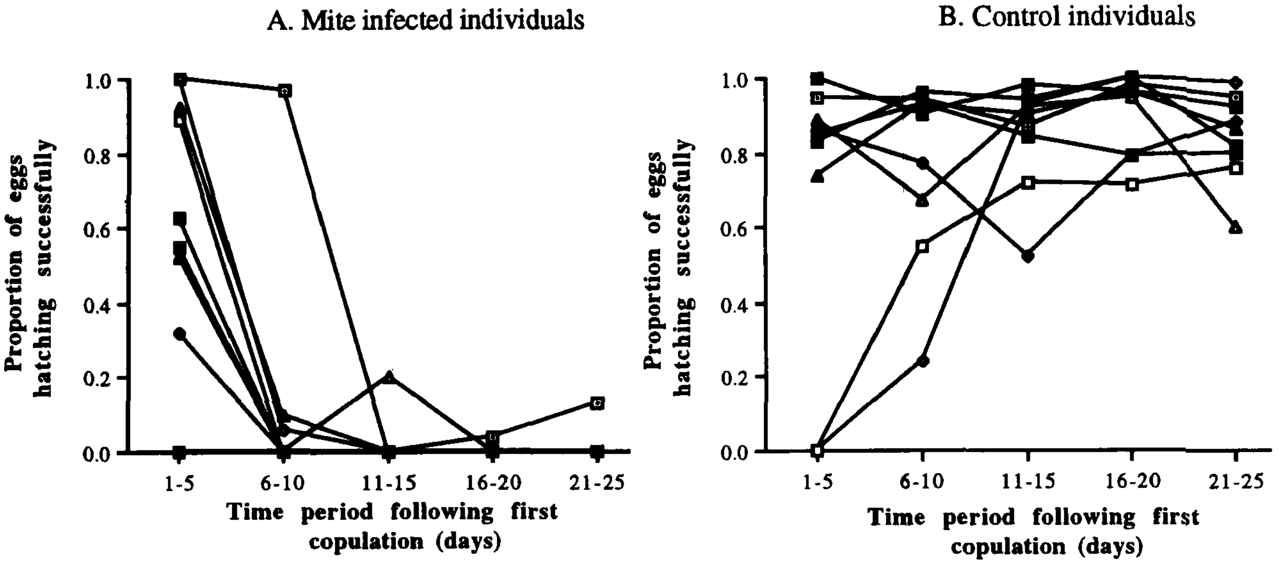

Fig. 1. Hatch-rate of eggs laid by female *A.bipunctata* bearing mites (A) and individuals free from infection (B) during sequential 5-day periods.

on the same alternating daily four aphid–three aphid ration. After 7 days the females were mated, and the fertility of the egg clutches produced monitored in comparison to the fertility of eggs from uninfected siblings on a similar regime. Each female was re-mated every 7 days to an uninfected male.

Results

The mites had a similar effect on the fertility of females from the Moscow population as those from the Cambridge population. Whereas the control individuals showed high egg hatch-rates throughout their lifespan, with at least 50% of the eggs hatching in every clutch, after on average 9.5 days (SE 1.0), all the eggs in clutches laid by the seven infected females shrivelled rapidly and failed to hatch, although yellow and intact when first laid.

Discussion

The mite *Coccipolipus hippodamiae* is transmitted between *A.bipunctata* partners during copulation. Tests show this infection to have a detrimental effect upon the lifetime reproductive success of adult females which bear it. The mite appears to produce roughly a 25% decrease in the number of eggs laid by infected ladybirds. In addition, the mite produced a massive decrease in the viability of the eggs laid by their host. The finding of a similar reduction in fertility and egg viability for infected females from the Moscow population showed that this effect is not a result of introduction of the mite to a previously uninfected host population, but a real effect of the mite on *A.bipunctata* in general. Therefore *A.bipunctata* may be considered to have a sexually transmitted disease of unusual severity.

The effect of the mite on males is more difficult to ascertain. The most probable effect would be a general reduction in vitality and vigour which would reduce the ability of males to locate and attract partners, but this would be difficult to quantify under the artificial conditions of the laboratory. However, even if the mite does not have any direct negative fitness effect on male hosts, it will still reduce their reproductive success, as it would be transmitted to their partners, and thus reduce their fertility.

Our experiments show that *A.bipunctata* from Moscow has a sexually transmitted mite which has negative fitness effects on its host. Is mite transmission confined to periods of mating? The experiments above revealed a low level of transmission to non-sexual-partner hosts (Table 1). The question arises as to whether this is an artefact of laboratory conditions. There are two reasons to think it is. First, ladybird density is much lower in the field than in the confined experimental boxes. Observations of the mite suggest it has limited longevity away from the host: mites placed in clean petri dishes in the laboratory live less than 12 h (Sharpe & Hurst, pers. obsn). Therefore mites leaving the host may have a very low probability of finding a new host. Second, the density and behaviour of larval mites on the hosts in the sexual transfer experiments are probably different from those observed on adults in the field, because the ladybirds were confined in single-sex dishes between experiments. If transfer of mite larvae takes place mainly during copulation, maintenance in single-sex dishes might produce unusually high mite densities upon the host. Furthermore, as the number of larval mites builds up on unmated adults, mites are seen to crawl over the surface of the ladybird and into the petri dish (Hurst, pers. obsn). Therefore, using rarely mated adults might increase mite transfer rates to non-sexual-partner ladybirds. However, although the experiments may have overestimated the amount of transmission between non-mating motile ladybirds, as found in spring or summer, the mite may transmit non-sexually during periods of diapause or aestivation, when they often aggregate (Majerus, 1991, 1994b). As yet it is impossible to say whether sexual transmission is sufficient in itself to maintain the infection in the population, or whether horizontal transmission unassociated with sex is also necessary and important.

The above study also provokes questions as to the cause of female sterility. It is hard to believe that the eeg inviability is a direct effect of the energy sap that the mite represents, given the modest decrease in the number of eggs laid. There are perhaps two possibilities. First, that the mite either uses or interferes with the production of a particular nutrient required for host egg fertility. The collapse of eggs laid by mite-infected individuals is perhaps symptomatic of an ineffective chorion; removal of this layer is known to produce egg death by drying (Ricci & Stella, 1988). Alternatively, the effect may not be due to the mite directly, but due to pathogenic micro-organisms which it introduces to its host. Insect resistance to infection comes largely from the gut and cuticle barrier, and parasitoid wasps have been recorded as vectors of a diverse array of pathogenic micro-organisms, from microsporidia of the genus *Nosema* (Siegel *et al.*, 1986), to viruses (Irabagon & Brooks, 1974; Beegle & Oatman, 1975) and bacteria (Bucher, 1963). Ectoparasitic mites may cause similar disease transfer, and represent an important source of new infections.

Perhaps the most important sphere in which this study has importance is in discussions of the population biology of infectious diseases in invertebrates. This disease affects only adults, and sexual transmission appears to be of key importance in its maintenance (but see caution above). The case is similar to the peculiar case of the sexually transmitted ectoparasitic nematode of noctuid moths. Such diseases must be expected to be rare in invertebrates, for as well as requiring host promiscuity (which is certainly true of *A.bipunctata*: Majerus, 1994a), they also require either significant overlap of generations, with inter-generational matings, or an alternate host to exist. Without an alternate host, years in which no overlap of generations occurs would cause local extinction of the sexually transmitted organism. This is perhaps where the podapolipid mites represent a unique case. Whereas *Noctuidonema*, the sexually transmitted nematode, is found on many species of noctuid moths (Remillet & Silvain, 1988; Rogers *et al.*, 1990a, b), *Coccipolipus* species are known to be very limited in their host range within coccinellid beetles (e.g. Schröder, 1979). Whereas in *Noctuidonema*, other species may act as a pool for re-infection following local extinction, the paucity of alternate hosts for members of the genus *Coccipolipus* suggests a greater reliance on overlap of host generations. The importance of overlap of generations is also consistent with the limited observations of *C.hippodamiae*. It is known to be present in Moscow, but not Cambridge. In Britain, *A.bipunctata* has one main generation per year, with a partial second generation in many years produced partly by inter-generational pairings (Majerus, 1994b). However, from time to time inter-generational crosses are prohibited because the over-wintering generation dies out before their progeny reach reproductive maturity. In Moscow, however, three generations of *A.bipunctata* may be observed in a year (Zakharov, pers. obsn).

The finding of a sexually transmitted organism also merits consideration in an evolutionary light. The presence of an STD may be expected to have an evolutionary effect on host sexual behaviour (Borgia & Collis, 1989; Hamilton, 1990; Sheldon, 1993). The study of mate choice in *A.bipunctata* has a long history (see Ritchie, 1992, for review). The presence of an STD adds an extra dimension to this study. Females which can avoid mating with infected males will clearly be favoured by natural selection, as may male selection of uninfected females. An elaborate mate rejection behaviour is observed in *A. bipunctata*: a female may prevent intromission by pulling her abdomen up under her elytra, and may use various physical means (rolling over, kicking, raising legs, running (Majerus, 1994b)) to displace an unwanted suitor. Observations of initial stages of interaction between male and female, and later possible rejection behaviour, suggest that the female may be testing the male's vigour (see Majerus, 1994b, for review). If this is true, then it may be that the interaction between male and female is the evolutionary product, in part, of the need to avoid disease. Mate choice may, in part, derive from the need to avoid infection with parasites (Borgia & Collis, 1989). Clearly, this awaits test.

The evolutionary effect of this mite on its host may go beyond the design of mating behaviour. Mites have been considered a potential source of novel infections with transposable elements and inherited symbionts, these having only a short life outside the host (Houck *et al.*, 1991). *Adalia bipunctata* is host to inherited parasites, a bacterium of the genus *Rickettsia* (Hurst *et al.*, 1992, 1993; Werren *et al.*, 1994), and one of the genus *Spiroplasma* (Hurst *et al.*, 1995). It is notable that members of the Acari do bear a *Spiroplasma* similar to that which causes male-killing in Moscow *A.bipunctata* (Tully *et al.*, 1981; Weisburg *et al.*, 1989). Could a female ladybird be infected with a micro-organism following parasitization by a mite and transmit that micro-organism to its progeny? Significant here is that, although egg fertility decreases following mite infection, some progeny may be produced to pass on novel inherited parasites. Mite infection does not always reduce fertility to zero, and *A.bipunctata* females have been observed to recover after 80 days of infection, due to the death of the mite colony (Hurst, pers. obsn). This might allow the transmission of any novel bacterial infection to progeny. Clearly, work on the role of mites such as the podapolipids in vectoring inherited parasites (both bacteria and transposable elements) would be timely, in this and other systems.

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