No evidence that presence of sexually transmitted infection selects for reduced mating rate in the two spot ladybird, *Adalia bipunctata*

Sophie L Jones, Daria Pastok, Gregory D D Hurst

Sexually transmitted infections (STIs) are common in animals and plants, and frequently impair individual fertility. Theory predicts that natural selection will favour behaviours that reduce the chance of acquiring a STI. We investigated whether an STI, *Coccipolipus hippodamiae* has selected for increased rejection of mating by female *Adalia bipunctata* as a mechanism to avoid exposure. We first demonstrated that rejection of mating by females did indeed reduce the chance of acquiring the mite. We then examined whether rejection rate and mating rate differed between ladybirds from mite-present and mite-absent populations when tested in a common environment. No differences in rejection intensity or remating propensity were observed between the two populations. We therefore conclude there is no evidence that STIs have driven the evolution of female mating behaviour in this species.

1   **No evidence that presence of sexually transmitted infection selects for reduced**

2   **mating rate in the two spot ladybird, *Adalia bipunctata*.**

4   Sophie L Jones(+), Daria Pastok(+) & Gregory D D Hurst (1)

5   Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK.

6   1. For correspondence: g.hurst@liv.ac.uk +44 151 7954520

7   + Contributed equally to the work.

9 **Abstract**

10 Sexually transmitted infections (STIs) are common in animals and plants, and frequently impair

11 individual fertility. Theory predicts that natural selection will favour behaviours that reduce the

12 chance of acquiring a STI. We investigated whether an STI, *Coccipolipus hippodamiae* has

13 selected for increased rejection of mating by female *Adalia bipunctata* as a mechanism to avoid

14 exposure. We first demonstrated that rejection of mating by females did indeed reduce the

15 chance of acquiring the mite. We then examined whether rejection rate and mating rate differed

16 between ladybirds from mite-present and mite-absent populations when tested in a common

17 environment. No differences in rejection intensity or remating propensity were observed between

18 the two populations. We therefore conclude there is no evidence that STIs have driven the

19 evolution of female mating behaviour in this species.

INTRODUCTION

Sexually transmitted infections (STIs) can be defined as infections that are primarily transmitted following sexual contact. Over 200 STIs have been identified to date and have been discovered in 48 families and 27 orders of hosts (Lockhart et al., 1996). Hosts vary from plants (e.g. white campion *Silene alba* suffers from infection of the pollinator-transmitted anther smut *Ustilago violacea* (Thrall et al., 1993)), through to mammals (e.g. horses can be infected by *Trypanosoma equiperdum* (Smith and Dobson, 1992)). In the past, vertebrate STIs were the most heavily studied and widely understood STIs, and insect STIs were somewhat neglected (Smith and Dobson, 1992, Sheldon, 1993, Lockhart et al., 1996, Lombardo, 1998). However in more recent years, insect STIs have received increasing attention. Knell & Webberley (2004) noted records of 73 species of STIs infecting approximately 182 species of insect. Insect STIs recorded to date are most commonly multicellular ectoparasites, such as mites, worms and fungi.

Most STIs have relatively small negative effects on host mortality, but tend to reduce fecundity or sterilise the host (Lockhart et al., 1996). Natural selection should therefore favour host traits that reduce the risk of infection. There are three possible behavioural routes to reducing the chance of acquiring an STI. First, if female fertility is not limited by low remating rates, exposure can be limited by mating with fewer partners. Theory predicts that STI presence should select for an increase in female refusal to mate when courted (Boots and Knell, 2002, Kokko et al., 2002). Second, there is the possibility of rejection of infected partners in favour of uninfected ones. Whilst there is some evidence for contagion avoidance choices for 'classic' infections (Able, 1996), studies to date have failed to find evidence for avoidance of mating with individuals carrying an STI (Abbot and Dill, 2001, Webberley et al., 2002, Nunn, 2003). This distinction may be associated with the strong selection on STIs to be cryptic to enable

44   transmission (Knell, 1999). Finally, it has been postulated that some post-copulatory grooming

45   processes, and in cape ground squirrels, post-copulatory masturbation, may have evolved as a

46   means of preventing STI transmission (Hart et al., 1988, Nunn, 2003, Waterman, 2010).

47   The interaction between the two-spot ladybird, *Adalia bipunctata*, and its ectoparasitic mite

48   *Coccipolipus hippodamiae*, represents one of the best studied invertebrate-STI interactions. The

49   mite lives under the elytra of the beetle, and larval mites move between host individuals that are

50   copulating (Hurst et al., 1995). Mite infection in females is associated with a rapid loss of

51   fertility, such that acquiring an infection is very costly to females. The two-spot ladybird is a

52   promiscuous species where females mate once every 2-3 days in the wild (Haddrill et al., 2008).

53   Where the mite is present, this promiscuity leads to an epidemic of this disease during the

54   spring/summer mating season, during which nearly all adult beetles become infected (Webberley

55   et al., 2006a, Ryder et al., 2013, Ryder et al., 2014).

56   The STI is thus both prevalent and highly costly to female hosts, creating a selection pressure for

57   direct avoidance of infected partners through mate choice, and indirect avoidance of mite

58   acquisition through reduced mating rate. Previous laboratory and field studies provided no

59   evidence that ladybirds discriminated against infected partners in mating decisions (Webberley et

60   al., 2002). However, the hypothesis that selection has acted to increase the general tendency to

61   reject matings has not been tested. One prediction of this hypothesis is that rejection behaviour

62   should be more intense, and mating rate lower, in ladybirds from populations where the mite is

63   present.

64   In this paper, we examine first whether rejection is efficient at preventing mite transfer, and then

65   test the hypothesis that ladybirds from populations in which the STI is present have been selected

66   for more intense rejection behaviour and lower mating rate, as a means of avoiding infection.

67   Our measures, which are made under standardized laboratory conditions, provide no evidence

68   that rejection behaviour or remating propensity differs between these populations.

69

70   MATERIALS AND METHOD

71

72   *Experiment 1: Is rejection of mating by a female an efficent means of preventing transmission of*

73   *C. hippodamiae infection?*

74   Female and male ladybirds were collected from Stockholm in June/July 2011 and returned to the

75   laboratory. They were sexed and classified as being uninfected, latent infected or infectious on

76   the basis of absence of mites, presence of mites without infectious larval mites, and presence of

77   larval mites ready to transmit. Pairs comprising a single infectious male with a focal uninfected

78   female, and single infectious female with a focal uninfected male were established in clean 90

79   mm in diameter Petri dishes in the laboratory, and behaviour observed for 30 minutes. Behaviour

80   was scored as no interaction, rejected mating, and successful mating. Pairs that mated were

81   allowed to mate to completion before separation of the focal partner to a new dish. The focal

82   individual was then examined 24 hour later for the presence of larval mites, and where present,

83   the number of larval mites was scored. The importance of focal host sex and mating/rejection on

84   mite transfer was analysed with a binomial GLM.

85

86   *Experiment 2: Do female beetles from populations that carry the STI show lower mating rates*

87   *and a greater likelihood of rejecting mating?*

88   *Adalia bipunctata* were collected from two locations c. 300 km apart in Sweden during August

89   2012: Nässjö (57.7°N, 14.7° E) and Stockholm (59.3°N, 18.1°E). The Nässjö population is free of

90   mite infection (Webberley et al., 2006b), whereas there is an annual epidemic of the infection in

91   Stockholm, leading to nearly all beetles becoming infected (Ryder et al., 2013, Ryder et al.,

92   2014). Females from these populations were allowed to mate with sympatric males, and progeny

93   reared in the laboratory. This rearing was conducted concurrently for both populations to

94   standardize environment. The resulting adult ladybirds were sexed and maintained in single sex

95   dishes with an ample supply of pea aphid food for 30 days, creating ladybirds of equivalent

96   reproductive maturity to that seen in the May/June mating period. These ladybirds were then

97   used before experimental analysis of rejection behaviour and mating rate. All behavioural

98   observations occurred in the absence of mites to avoid any direct impact of mites on the mating

99   behaviour of their host (although none have previously been observed: (Webberley et al., 2002)).

100

101   Rejection behaviour and mating rate were analysed over daily mating trials carried out over a

102   five day period. 'Pools' of five females and five males were created for each population. In each

103   case males were from same population as females, but unrelated to them. Within each pool,

104   males and females were mixed and allowed to mate once three days before the experiment. This

105   was intended to reduce artefactual behaviour resulting from single sex confinement.

106   Subsequently, females from each pool were offered a male for 30 minutes at the same time each

107   day for a five day period, with each female being offered a different male every day (see Table 1

108   for block design).

109   During each mating trial, each pair was placed in a clean Petri dish at 21°C for the duration of the

110   observation, and the presence of the following behaviour observed:

111   a) The number of interactions between male and female

112   b) The presence and duration of rejection behaviour during these interactions. Rejection

113   behaviour was categorised into different intensity levels; no rejection observed; mild

114   rejection (<1 minute); moderate rejection (1-5 minutes) and intense rejection (>5

115   minutes).

116   c) Whether interactions resulted in mating

117   From these measures, the likelihood of a female rejecting mating, the intensity of rejection, and

118   the probability of successful mating occurring were calculated.

119   Four replicate groups were used, resulting in 20 females being tested for each population.

120

122   RESULTS
123

124   *Experiment 1: Is rejection of mating by a female an efficent means of preventing transmission of*

125   *C. hippodamiae infection?*

126   Transmission rates from wild caught infectious male and female individuals to uninfected

127   partners with which they mated were high, with only one of 26 females not acquiring infection

128   during mating with an infectious male partner, and one of 35 males not acquiring infection from

129   an infectious female partner. In contrast, transmission was rare when mating was rejected, with

130   one of seven females acquiring an infection following rejection of the infectious male, and one of

131   three males acquiring infection having been rejected by an infectious female. Statistical analysis

132   revealed no evidence for an interaction term between sex of infected host and mating/rejection

133   behaviour on mite transfer probability. Statistical analysis with the interaction term dropped

134   revealed no effect of donor sex on transmission probability (GLM factor host sex, p=0.288), but

135   a significant effect of the factor 'rejected/mated' (GLM factor mated/rejected, p<0.0001). Thus

136   rejection behaviour by the female is protective against mite transfer both from an infected male,

137   and additionally prevents transmission to an uninfected male partner. We additionally examined

138   the number of larval mites transferred during copulation /rejected copulation for the cases where

139   larval mites were transferred. The intensity of infection following the two rejected matings where

140   mites did transfer was low (1 and 2 larval mites) compared to that observed for completed

141   pairings (median 10, range 2-30, n=56).

142

143   *Experiment 2: Do female beetles from populations that carry the STI show a greater likelihood*

144   *of rejecting mating and a lower mating rate?*

145   Mating was observed to be more common on day 1 than on other days in experiments involving

146   both Stockholm and Nässjö (Figure 1). We pooled mating trial outcome data across repeats and

147   populations, and observed that mating rate was heterogeneous between days within the

148   experiment (χ^2=16.042, df=4, p=0.003). This heterogeneity is associated with high mating rates

149   on day 1 (after 3 days without mating activity); when day 1 is excluded, mating rates are

150   homogenous over days 2-5 (χ^2=0.276, df=3, p=0.964). Thus, in further analysis, day 1 mating is

151   excluded, as the high mating rate on this day is likely to be associated with experimentally

152   induced lack of mating opportunity.

153   We then examined whether there was any evidence for a difference in mating behaviour between

154   the two populations from days 2-5. We pooled all encounters, and analysed the outcome of the

155   80 male-female interaction trials in each population. We observed that males approached females

156   for mating in 64 cases for both populations. Where interactions occurred, most females exhibited

157   some rejection behaviour in encounters, and this rejection was prolonged in over half of cases in

158   both populations. There was no evidence that females from the two populations differed in the

159   intensity of rejection behaviour following a male's attempt to mate (χ^2=4.13, df=3, p=0.25)

160   (Figure 3).

161   There was also no evidence for variation in overall propensity to mate between ladybirds from

162   Nässjö (mite free in nature) and Stockholm (mite present in nature) (Figure 2). Across days 2-5,

163   there was no evidence of an association between population and remating rate (χ^2=0.627, df=1,

164   p=0.428). We additionally reanalysed mating propensity to create a more ecologically relevant

165   statistic. The confined experiment of the Petri dish allows males the ability to interact with

166   female repeatedly, which is unlikely to occur in the field. An 'environmental' mating rate based

167   on the result of the first interaction between male and female only was therefore calculated,

168   which discounted mating if this took more than five minutes to achieve. The 'environmental'

169   mating rate for Stockholm and Nässjö was half that of the overall mating rate (Figure 3).

170   Analysis indicates there was no evidence of association between location and 'environmental'

171   mating rate (χ^2=0.295, df=1, p=0.587).

172   DISCUSSION

173   Sexually transmitted infections are common in nature, and are frequently harmful to female hosts

174   (Lockhart et al., 1996). Models predict that the presence of STIs should therefore select on

175   female mating behaviour. Past work has failed to reveal any choice of mates associated with STI

176   avoidance (Abbot and Dill, 2001, Webberley et al., 2002, Nunn, 2003). However, there has been

177   no test of the hypothesis that selection will promote avoidance of STIs through reducing mating

178   rate (Boots and Knell, 2002, Kokko et al., 2002). In this study, we first studied the impact of

179   rejection behaviour on mite transfer. We observed rejecting mating was protective against mite

180   transfer, with a reduced probability of transmission during rejected mating. Further, where mite

181   transmission occurred, a lower number of larval mites transferred during copulation, and low

182   intensity initial infections such as these are less like to develop into mature infection (Pastok et

183   al., 2015). Thus, we can conclude rejection of mating by females would be protective, and

184   selection on females to reject mating would be predicted.

185   In contrast to this, we failed to observed differences in female tendency to reject matings when

186   beetles from Stockholm (where the STI is naturally present) and Nässjö (which is naturally

187   uninfected) were compared. No evidence was found for differences in tendency to attract

188   courtship, nor in the presence or intensity of rejection behaviour exhibited by females when

189   contacted by a male, nor in the overall outcome measured in terms of mating/not mating.

190   Combined with previous observations of lack of mate choice for uninfected partners, the data do

191   not support the hypothesis that STIs have selected on female mating behaviour in this species,

192   despite rejection of mating being partly effective at preventing STI transmission.

193   Failure to find a difference in mating rates between the two populations could have four sources.

194   First, there may be no difference. Second, there may be a difference but the effect size is small.

195   However, we would note that mating rate was quantitatively higher in beetles from Stockholm

196   (mite present population) than Nässjö (mite absent). Third, the beetles in the experiment may not

197   fully represent the populations they derive from. Whilst the beetles used in each repeat of the

198   experiment were outbred and different individuals, they derived from 5 families in each case.

199   The sample is an estimate of the individuals in the population they derive from, rather than fully

200   representing the populations. This would not affect our ability to uncover fixed differences

201   between populations. It would, however, potentially compromise our ability to detect the

202   evolution of a mixed risky/safe strategy in response to STI presence, as suggested by Boots &

203   Knell (Boots and Knell, 2002, Kokko et al., 2002). Fourth, the behaviour is observed in the

204   laboratory, removed from natural conditions. 'Naturalness' is always a problem for laboratory

205   study. Despite an experimental design that attempted to replicate natural mating environment e.g.

206   temperature, lighting, there were possible critiques of spatial confines, repeated interaction and

207   ineffective behaviour. However, consideration of the first interaction only did not alter the

208   conclusion that the outcome of male/female interactions did not vary between populations. Thus,

209   it is currently most parsimonious to conclude there are no fixed biological differences in mating

210   propensity between these two populations.

211   We are thus confident that the presence of a sterilizing STI that reaches high prevalence has not

212   led to the evolution of increased female rejection behaviour. Why has an intuitive evolutionary

213   path not been taken? One possibility is that a high mating rate is required for female fertility,

214   such that females who refuse to mate incur a cost. However, *Adalia* females mated singly have

215   equivalent fertility, measured over 20 days, to females mated every two days (Haddrill et al.,

216   2007). Thus, there is ample scope for a female's risk of mite induced infertility to be reduced

217   before sperm-depletion associated infertility is observed. A second possibility is that local

218   adaptation is not possible in this species, or that there has not been sufficient time for adaptation

219   to occur. The presence of variation in the frequency of colour pattern variants in this species on

220   equivalent spatial scales (Brakefield, 1984) make us confident gene flow is not sufficient to

221   impede local adaptation. Historical records of mites on European ladybirds dating back 20 years

222   indicates this is not a very recent interaction, and thus we do not believe that the lack of a

223   response is associated with evolutionary lag. A third hypothesis is that selection to prevent STI

224   acquisition does operate in the way expected, but there are other factors differing between the

225   populations that influence mating rate evolution. It is possible that there is a counterbalancing

226   selective force working in opposition to the effect of the STI (e.g. spatially varying benefits of

227   polyandry). The source of such selection is not obvious (the two populations use similar habitat

228   and have similar sex ratio), but such a hypothesis cannot be ruled out. It is also possible that

229   there is a different, but hitherto cryptic, STI present in Nassjo. The presence of confounding

230   processes can only be properly excluded by a wider comparison of STI present/absent

231   populations, which would reduce the influence of any local confounding variables. Finally, the

232   prediction that STIs select for lower mating rate applies to female hosts, in which there are

233   smaller benefits to each additional mating, and in this species, higher costs of infection (sterility).

234   Selection on males is not expected to act in the same way, as each mating provides significant

235   fitness benefits, and the STI is only weakly costly to male hosts (Ryder et al., 2007). If mating

236   rate is determined by males, then the STI is less likely to drive mating system evolution.

237   In summary, our experiment demonstrated rejection behaviour was efficient at preventing STI

238   transmission, but did not occur more commonly in beetles derived from populations where the

239   STI was common. This study, combined with previous analysis indicating STI infected beetles

240   were not disadvantaged in acquiring mates (Webberley et al., 2002), produces no support for the

241   hypothesis that female mating behaviour evolves in response to the presence of a sterilizing STI.

242   An intriguing possibility is that STIs are most commonly observed in species in which evolution

243   to resist STI transmission is inhibited.

244   ACKNOWLEDGEMENTS. We wish to thank Tom Price for comments on the manuscript, and

245   Tom Heyes for technical support.

246

247   REFERENCES

248   ABBOT, P. & DILL, L. M. 2001. Sexually transmitted parasites and sexual selection in the
249   milkweed leaf beetle, *Labidomera clivicollis*. *Oikos,* 92**,** 91-100.
250   BOOTS, M. & KNELL, R. J. 2002. The evolution of risky behaviour in the presence of a
251   sexually transmitted disease. *Proc. R. Soc. Lond. B,* 269**,** 585-589.
252   BRAKEFIELD, P. M. 1984. Ecological studies on the polymorphic ladybird *Adalia bipunctata*
253   in the Netherlands. II Population dynamics, differential timing of reproduction and
254   thermal melanism. *J. Anim. Ecol.,* 53**,** 775-790.
255   HADDRILL, P. R., SHUKER, D. M., AMOS, W., MAJERUS, M. E. N. & MAYES, S. 2008.
256   Female multiple mating in wild and laboratory populations of the two-spot ladybird,
257   Adalia bipunctata. *Molecular Ecology,* 17**,** 3189-3197.
258   HADDRILL, P. R., SHUKER, D. M., MAYES, S. & MAJERUS, M. E. N. 2007. Temporal
259   effects of multiple mating on components of fitness in the two-spot ladybird, Adalia
260   bipunctata (Coleoptera : Coccinellidae). *European Journal of Entomology,* 104**,** 393-398.

261 HART, B. L., KORINEK, E. K. & BRENNAN, P. L. 1988. POSTCOPULATORY
262   GROOMING IN MALE-RATS PREVENTS SEXUALLY-TRANSMITTED
263   DISEASES. *Annals of the New York Academy of Sciences,* 525**,** 397-398.
264 HURST, G. D. D., SHARPE, R. G., BROOMFIELD, A. H., WALKER, L. E., MAJERUS, T. M.
265   O., ZAKHAROV, I. A. & MAJERUS, M. E. N. 1995. Sexually transmitted disease in a
266   promiscuous insect, *Adalia bipunctata. Ecol. Entomol.,* 20**,** 230-236.
267 KNELL, R. J. & WEBBERLEY, K. M. 2004. Sexually transmitted diseases of insects:
268   distribution, ecology, evolution and host behaviour. *Biol. Rev.,* 79**,** 557-581.
269 KOKKO, H., RANTA, E., RUXTON, G. & LUNDBERG, P. 2002. Sexually transmitted disease
270   and the evolution of mating systems. *Evolution,* 56**,** 1091-1100.
271 LOCKHART, A. B., THRALL, P. H. & ANTONOVICS, J. 1996. Sexually transmitted diseases
272   in animals: ecological and evolutionary implications. *Biol. Revs.,* 71**,** 415-471.
273 LOMBARDO, M. P. 1998. On the evolution of sexually transmitted diseases in birds. *Journal of*
274   *Avian Biology,* 29**,** 314-321.
275 NUNN, C. L. 2003. Behavioural defenses against sexually transmitted diseases in primates.
276   *Animal Behaviour,* 66**,** 37-48.
277 PASTOK, D., ATKINSON, D. A. & HURST, G. D. D. 2015. Assessing the impact of male-
278   killing bacteria on the spread of a sexually transmitted infection. *Animal Behaviour,* in
279   press.
280 RYDER, J. J., HATHWAY, J. & KNELL, R. J. 2007. Constraints on parasite fecundity and
281   transmission in an insect-STD system. *Oikos,* 116**,** 578-584.
282 RYDER, J. J., HOARE, M.-J., PASTOK, D., BOTTERY, M., BOOTS, M., FENTON, A.,
283   ATKINSON, D., KNELL, R. J. & HURST, G. D. D. 2014. Disease Epidemiology in
284   Arthropods Is Altered by the Presence of Nonprotective Symbionts. *The American*
285   *Naturalist,* 183**,** E89-E104.
286 RYDER, J. J., PASTOK, D., HOARE, M.-J., BOTTERY, M. J., BOOTS, M., KNELL, R. K.,
287   ATKINSON, D. & HURST, G. D. D. 2013. Spatial variation in food supply, mating
288   behavior, and sexually transmitted disease epidemics. *Behavioral Ecology,* 24**,** 723-729.
289 SHELDON, B. C. 1993. Sexually transmitted disease in birds: occurence and evolutionary
290   significance. *Phil. Trans. R. Soc. Lond. B,* 339**,** 491-497.
291 SMITH, G. & DOBSON, A. P. 1992. Sexually transmitted diseases in animals. *Parasitology*
292   *Today,* 8**,** 159-166.
293 THRALL, P. H., BIERE, A. & ANTONOVICS, J. 1993. Plant life-history and disease
294   susceptibility-the occurence of *Ustilago violacea* on different species within the
295   Caryophyllaceae. *J. Ecol.,* 81**,** 489-498.
296 WATERMAN, J. M. 2010. The Adaptive Function of Masturbation in a Promiscuous African
297   Ground Squirrel. *PLoS ONE,* 5**,** e13060.
298 WEBBERLEY, K. M., BUSZKO, J., ISHAM, V. & HURST, G. D. D. 2006a. Sexually
299   Transmitted Disease Epidemics in a Natural Insect population. *J. Anim. Ecol.,* 75**,** 33-43.
300 WEBBERLEY, K. M., HURST, G. D. D., BUSZKO, J. & MAJERUS, M. E. N. 2002. Lack of
301   parasite-mediated sexual selection in a ladybird/sexually transmitted disease system.
302   *Anim. Behav.,* 63**,** 131-141.
303 WEBBERLEY, K. M., TINSLEY, M. C., SLOGGETT, J. J., MAJERUS, M. E. N. & HURST,
304   G. D. D. 2006b. Spatial variation in the incidence of a sexually transmitted parasite of the
305   ladybird beetle Adalia bipunctata (Coleoptera : Coccinellidae). *European Journal of*
306   *Entomology,* 103**,** 793-797.

307

309

Experimental design for mating experiment, indicating rotation of partners within block

Table 1: Five day experimental block design of sympatric matings between Stockholm (SF1=Stockholm Female 1, SM1=Stockholm Male 1 etc) and Nässjö (NF1= Nässjö Female 1, NM1= Nässjö Male 1 etc.) individuals. Numbers in the matrix indicate day of mating.

	SF1	SF2	SF3	SF4	SF5
SM1	5	4	3	2	1
SM2	1	5	4	3	2
SM3	2	1	5	4	3
SM4	3	2	1	5	4
SM5	4	3	2	1	5

	NF1	NF2	NF3	NF4	NF5
NM1	5	4	3	2	1
NM2	1	5	4	3	2
NM3	2	1	5	4	3
NM4	3	2	1	5	4
NM5	4	3	2	1	5

Probability of mating for *A. bipunctata* from Stockholm (STI present population) and Nassjo (STI absent population) on each of five days

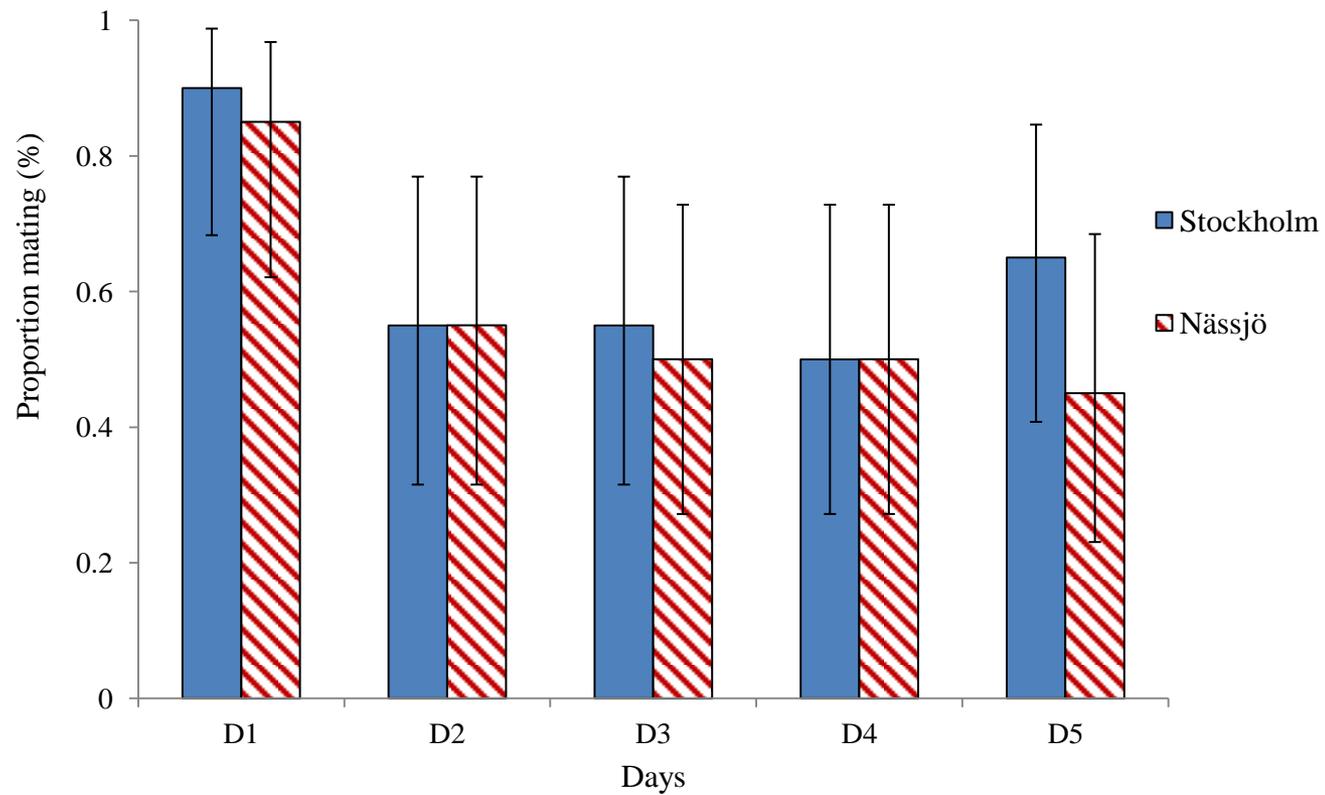
Figure 1: Proportion of pairs that mated each day during 30 minute period from Stockholm (Blue, STI naturally present in nature, though absent in the experiment) and Nässjö (Hatched Red, no STI). N=20 for all days, the combined results from four blocks. Error bars for proportionate data represent binomial sampling intervals calculated using the Clopper –Pearson (1934) method.

Rejection behaviour by female *A. bipunctata* from Stockholm (mite present) and Nassjo (mite absent) populations

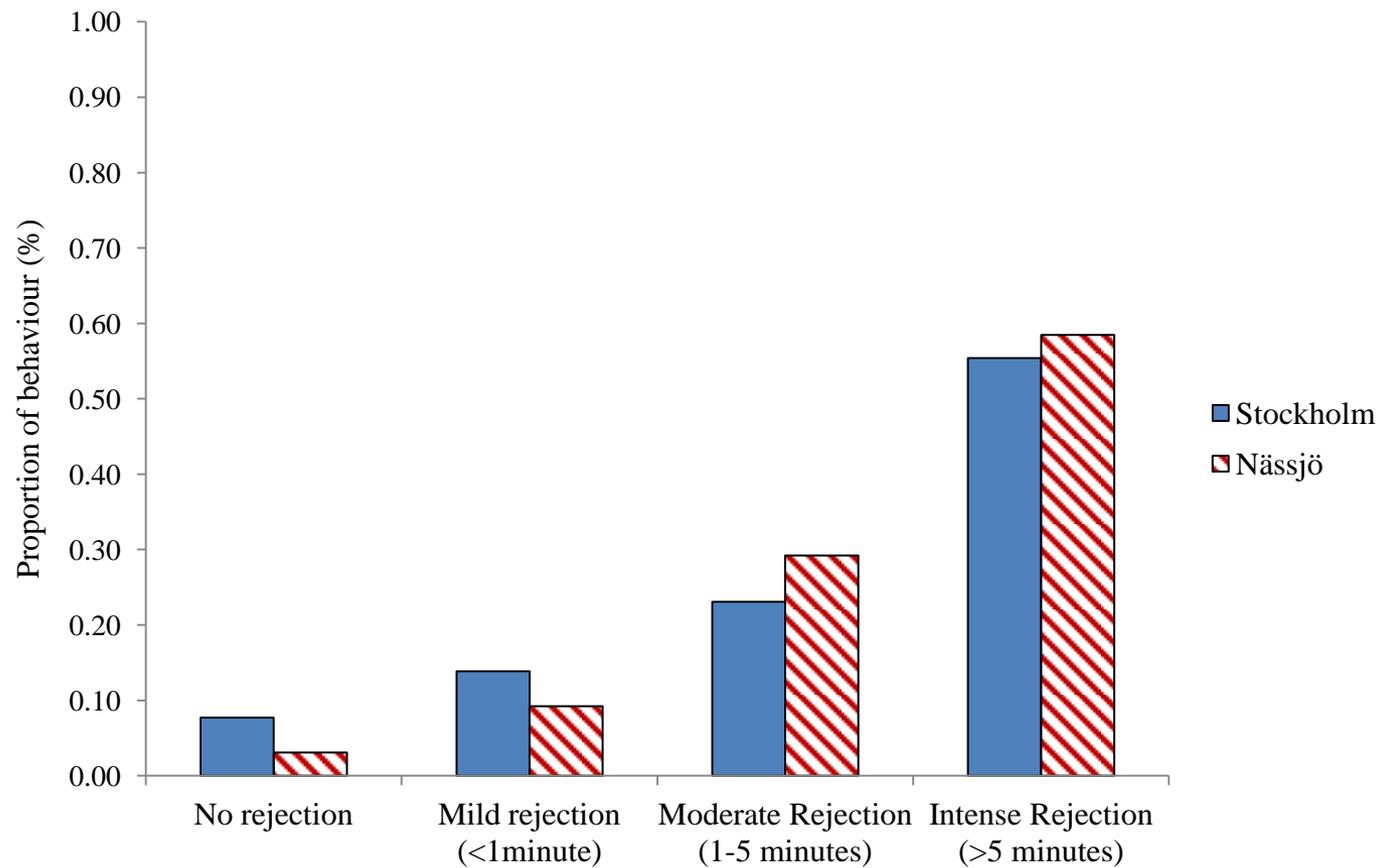
Figure 2: Proportion of different intensities of rejection behaviour (No rejection, mild rejection (<1minute), moderate rejection (1-5minutes), intense rejection (>5minutes)) observed from Stockholm (Blue, STI naturally present, though absent in the laboratory) and Nässjö (Hatched Red, no STI) females during 30 minute period experiments over days 2-5. N=64 for both populations.

Environmentally relevant mating rate for *A. bipunctata* from Stockholm (mite present population) and Nassjo (mite absent population).

Figure 3: 'Environmental' mating rate for Stockholm (Blue, STI naturally present, absent in the laboratory) and Nässjö (Hatched Red, no STI) ladybirds over days 2-5. A pair was considered to have mated only if the first interaction between male and female led to mating. N= 20 female beetles, 80 interactions, for both populations.