

1 **The role of host phenology in determining the incidence of an insect sexually transmitted**
2 **infection.**

3

4 **Daria Pastok (1), Mary-Jo Hoare (1), Jon J Ryder (1,2), Mike Boots (2), Rob J Knell (3), David**
5 **Atkinson (1), & Gregory D D Hurst (1 +)**

6 1. Institute of Integrative Biology, University of Liverpool, Crown Street Liverpool L69 7ZB, UK

7 2. Centre for Ecology & Conservation, Biosciences, College of Life & Environmental Sciences

8 University of Exeter, TR10 9EZ, U.K.

9 3. School of Biological and Chemical Sciences, Queen Mary, University of London

10 Mile End Road, London E1 4NS, U.K.

11 + for correspondence: g.hurst@liv.ac.uk +44 151 7954520

12

13 Abstract

14 Changes in the timing of life history events within the year alter the degree to which the
15 activity patterns of different species coincide, making the dynamics of interspecific
16 interactions sensitive to the phenology of the interacting parties. For parasites, the
17 availability of suitable hosts represents a crucial determinant of dynamics, and changes in
18 the host (and parasite) phenology may thus alter disease epidemiology and the conditions
19 for disease maintenance. We tested the hypothesis that the incidence of a sexually
20 transmitted mite infection, *Coccipolipus hippodamiae*, in *Adalia bipunctata* ladybird beetles
21 in Sweden was determined by host phenology, namely presence/absence of sexual contact
22 between cohorts of the host. We observed that the pattern of mite incidence in Swedish *A.*
23 *bipunctata* populations was consistent over time implying a constant biological/ecological
24 basis underlying the incidence. Further, ladybirds from populations where the mite was
25 absent were able to acquire mites during copulation, develop a mite infection, and transmit
26 infection onward, indicating an ecological (rather than biological) driver of mite incidence.
27 Observations of ladybird phenology in natural populations provided evidence of sexual
28 contact between the overwintered and new cohort adults that would permit mite transfer
29 between cohorts. In contrast, new cohort ladybirds in the two northern Swedish
30 populations where the mite was not present had not had sexual contact with the
31 overwintered generation, creating a 'hard stop' to mite transmission. We conclude that
32 variation in host phenology may be an important driver of the incidence of sexually
33 transmitted infections (STIs) by determining the presence/absence of sexual contact
34 between generations. More generally, we hypothesize that sensitivity to variation in host
35 phenology will be highest for parasites that infect one host species, one host life stage and
36 are directly transmitted on contact between host individuals.

37

38 Introduction

39 Phenological variation is now widely appreciated to be an important and general driver of
40 ecological interactions (Johansson et al. 2015), including those with natural enemies
41 (Thomson et al. 2010). In its most commonly considered form, differential alteration of the
42 timing of the activity of interacting species is considered as a driver of their contact rate,
43 and thus ecological and evolutionary dynamics. For parasite-host interactions, both theory
44 and experimental studies indicate the degree of match in the pattern of phenology of the
45 two parties impacts upon the dynamics of the interaction (Godfray et al. 1994, Paull and
46 Johnson 2014). For parasites with multiple host species, phenological shifts may additionally
47 alter the balance of host species utilized (Moller et al. 2011). A pressing challenge is to
48 determine the importance of phenological shifts as a driver of disease dynamics in natural
49 populations, both in terms of understanding spatial variation in interactions, and predicting
50 the influence of climate change on ecological dynamics.

51 Recent studies have suggested a potential role of phenological variation in driving the
52 incidence of sexually transmitted infections (STIs) of invertebrates (Hurst et al. 1995,
53 Seeman and Nahrung 2004). STIs are unusual in that they are confined to adults, and are
54 transmitted directly during host copulation. In many species of invertebrate, the adults may
55 exist in distinct cohorts – both in temperate environments associated with seasonal forcing,
56 but also in tropical insects that display ‘generation cycles’ (Godfray & Hassell, 1989; Knell
57 1998). As Knell & Webberley (2004) argue, infections that are solely transmitted during host
58 copulation can only be maintained where cohorts of adults overlap and there is inter-
59 generational mating that allows the parasite to transmit to the new cohort. A ‘pure’ STI
60 would disappear from a host population where there was a ‘generation gap’: periods where
61 no adult individuals are present, or where adults from one cohort have an obligatory
62 diapause before commencing mating activity. This provides the potential for phenological
63 changes in the host alone to drive the incidence of the STI. Despite the logic of this
64 argument, no empirical study to date has directly investigated the importance of host
65 phenology as a driver of STI/host dynamics (Knell and Webberley 2004).

66 In this study we assess whether phenological variation in the host drives the
67 presence/absence of an STI. The *Adalia bipunctata*/*Coccipolipus hippodamiae* two spot

68 ladybird/mite interaction provides an excellent test system for this hypotheses, as the basic
69 biology of the interaction and transmission processes are well established (Hurst et al.
70 1995), there is a good working knowledge of mite epidemiology within a cohort (Webberley
71 et al 2006a, Ryder et al. 2013, Ryder et al. 2014), and geographical variation in the presence
72 of the mite STI that has not been explained (Webberley et al. 2006b).

73 The mite lives under the elytra of its host, *A. bipunctata*, where it feeds on blood (Hurst et
74 al. 1995). Adult mites are sedentary, and produce eggs that hatch into motile larvae. These
75 larvae gather at tip of the elytra, and move between partners when they copulate, making
76 this an STI. The typical yearly cycle of the mite is well known from studies in Poland
77 (Webberley et al., 2006a; Ryder et al., 2013). The host species overwinters as an adult, and
78 1-10% of adult ladybirds that emerge from overwintering carry a mite infection. During May
79 and June, adult beetles mate every two days and oviposit to found the new cohort. The
80 mating activity results in a profound epidemic of the mite, such that nearly all overwintered
81 adult beetles still alive in July are infected (Webberley et al., 2006a). The speed of this
82 epidemic relates to factors such as food supply and population sex ratio, both of which
83 affect mating rate (Webberley et al 2006a, Ryder et al. 2013, Ryder et al. 2014). Mite
84 persistence then requires transmission from this cohort to the emerging adults of the new
85 generation, which emerges in July.

86 We here evaluate the role of phenological variation in driving mite presence/absence in
87 Swedish populations of *A. bipunctata*. Surveys between 2000 and 2002 revealed the mite
88 was present on *A. bipunctata* in three of four Swedish populations south of 61°N (Gävle,
89 Stockholm and Malmö) but was absent in all three populations north of 61°N latitude
90 (Webberley et al 2006b) (Figure 1a, Left hand panel). Our hypothesis was that absence of
91 mites from beetle populations would be associated with lack of opportunity for transmission
92 between cohorts, arising as a result of the overwintered cohort dying before sufficient new
93 cohort individuals emerged. To test this hypothesis, we studied three components. First, we
94 examined whether patterns of mite presence/absence were consistent over time, implying a
95 constant biological/ecological basis to infection incidence. Second, we tested the ability of
96 *A. bipunctata* from populations where the mite was absent to acquire mites during
97 copulation, develop a mite infection, and transmit infection onward. Finally, we examined
98 whether host phenological variation was associated with mite presence/absence.

100 1. *Pattern of C. hippodamiae* presence on *A. bipunctata* in Sweden in 2011 and 2012

101 Overwintered *A. bipunctata* were collected in 2011 and 2012 from Swedish urban habitats
102 (city centres and suburbs) of Malmö, Nässjö, Stockholm, Gävle, Ljusdal, Östersund and
103 Vilhelmina, mirroring locations previously sampled by Webberley et al. (2006b)(Figure 1a).
104 Ladybirds were collected from trees into a beating tray and from bushes by hand collection.
105 Collected individuals were transferred to 1.5 ml Eppendorf tubes. Ladybirds that were
106 caught as single individuals were confined singly, to prevent any contagion during storage. If
107 a mating pair landed on the beating tray, ladybirds were not separated but transferred
108 together into the tube.

109 Ladybirds were scored for mite presence within 24 hours of collection following Hurst et al.
110 (1995). To this end, each ladybird was carefully placed on its pronotum on Blu-tacTM and
111 examined under a binocular microscope. Each elytron was carefully unfolded using a pin to
112 expose the underside in turn, and the presence of mites was examined under x 40
113 magnification. The presence/absence of mite infection was noted, and if the infection was
114 present, the intensity of mite infection estimated (number of mite eggs, larvae, and adults).
115 An individual was deemed to be infected if any mite stage was present, and infectious if
116 adult and mite eggs and larvae were all present at the same individual. Mites were recorded
117 as present in a population where more than one ladybird individual in a sample carried
118 infection (this was to differentiate an established infection from ones associated with
119 occasional migrants). Records of mite presence/absence were compared to data from 2000-
120 2002 as recorded in Webberley et al. (2006b).

121 2. *Is absence of the mite from the north associated with inability of the parasite to*
122 *grow and transmit on northern ladybirds?*

123 We tested the ability of ladybirds from populations that did not carry mites naturally to
124 become infected with larval mites, develop infection and transmit the mite infection
125 onward. To this end, *A. bipunctata* were collected using a beating tray in Östersund and
126 Ljusdal (where the mite is absent) and in Stockholm (where the mite is naturally present) in

127 early August 2012. These ladybirds were then maintained in an incubator at 20°C with a
128 light regime of 20L:4D (20 hours of light and 4 hours of night), and fed aphids daily.

129 Thirteen uninfected ladybirds from Östersund (mites naturally absent), six uninfected
130 ladybirds from Ljusdal (mites naturally absent) and 19 uninfected ladybirds from Stockholm
131 (mites naturally present, control) were isolated individually in Petri dishes and paired with a
132 mite-infected partner (from Stockholm) and mating observed. The next day ladybirds were
133 separated from their infectious partner and then checked for the presence of larval mites
134 (successful initial transmission). Mite persistence and disease latent period (the time from
135 initial infection to infectious larval forms being produced) were then measured. To this end,
136 recipient ladybirds were checked for the progress of mite infection on the seventh,
137 fourteenth and seventeenth day post infection, and thereafter daily. Where infection
138 developed, the ability of the host to transmit mites onwards was tested. One week after the
139 ladybirds above were first scored as carrying larval mites (the infectious stage), they were
140 paired with an uninfected partner from the same population. Mating was observed as
141 before, and the recipient checked for larval mite presence the next day.

142 *3. Phenology of the host: sexual contact between generations and general observations*

143 We examined the degree of sexual contact between cohorts in detail through weekly
144 sampling in Stockholm (mite-present population) in 2010-2011, combined with a more
145 focussed analysis of whether new cohort beetles made sexual contact with old cohort
146 beetles in three mite-free and two mite-present populations in 2012. These directed
147 observations were combined with more general phenological information regarding the
148 timing of ladybird reproduction gained during survey work conducted in 2011.

149

150 Temporal sampling in Stockholm: We recorded the presence of overwintered and new
151 cohort beetles, and prevalence of mite infection in Stockholm (mite present population) in
152 weekly collections over the periods 17th May 2010-15th August 2010, and 21st May-27 July
153 2011. Beetles were collected by beating from lime trees in city centre and suburban
154 habitats, with aim of collecting 150/100 beetles (2010/2011 respectively), or if this number
155 of beetles could not be obtained, that which could be found in one hour. Individuals were
156 also noted as mating/single on collection. Collected beetles were sexed, scored for mite

157 presence, and scored as to cohort through elytral colour, with individuals belonging to an
158 overwintered generation being deep red/black, in contrast to the orange/red of adults from
159 the newly emerged generation (Majerus 1994).

160

161 Analysis of overlap between cohorts in mite free/mite present populations. From the
162 Stockholm data, it was clear that July and August represents the period when overwintered
163 and new generation adult ladybirds may be present together, a necessary condition for
164 transfer of the mite between generations. A break in the transmission cycle would occur
165 when new generation ladybirds emerged after the death of overwintered cohort. This would
166 be evidenced by the presence of virgin new cohort beetles in August with no overwintered
167 beetles present.

168 The co-occurrence of new and overwintered cohort adult ladybirds, and the matedness of
169 female new cohort ladybirds, was therefore examined in five different populations in August
170 2012 spanning the region where the mite showed presence/absence differentiation:

171 Östersund, Ljusdal (northern, both mite absent), Gävle, Stockholm (both mite present) and
172 Nässjö (southern, mite absent). Adult beetles were collected by beating lime tree branches
173 using a beating tray and by hand, and transported to the laboratory where they were scored
174 for sex, mite presence and for cohort. Alongside the collection of adult beetles, notes were
175 made on the presence of other life history stages in the population, as an indicator of the
176 timing of new cohort emergence.

177 The presence/absence of previous sexual activity of new cohort females from these samples
178 was then established through examining their fertility. Virgin female *A. bipunctata* lay eggs
179 at a low rate, such that lack of previous mating is indicated either by failure to lay eggs, or
180 failure to lay viable eggs. To this end 25 new cohort ladybird females from each site were
181 separated to individual Petri dishes. They were fed aphids daily. Eggs laid by females were
182 collected and kept in the incubator at 24°C. After 3-5 days eggs were scored as to whether
183 the eggs hatched (indicating prior mating) or became shrivelled (indicating no sperm store).
184 For females that did not lay viable eggs during this process, we then determined whether
185 they were competent to be fertile by permitting them to mate with males from their own
186 population, collecting eggs and assessing their viability.

187

188 General phenological information on the timing of ladybird reproduction. Notes were made
189 on the presence/absence of eggs, larvae and pupae on trees in May-July 2011 whilst
190 collecting beetles for the mite survey above. From these data, we estimated the time at
191 which oviposition began in the population as the earliest date of a) direct observation of
192 eggs b) observation of individuals in other life history phases, and then estimating the date
193 at which the eggs from which these individuals were derived would have been laid (using
194 the measure of egg-pupal development time of 21 days at 20°C in the laboratory, including 6
195 hour night time temperature of 12 C).

196

197

198

199 Results

200 1. *Pattern of C. hippodamiae* presence on *A. bipunctata* in Sweden in 2011 and 2012

201 Mite incidence showed a highly reproducible pattern of presence over space (Figure 1b
202 Right hand panel, Table S1). In brief, the mite was present in Malmö, Gävle and Stockholm
203 in all samples, but absent in Nässjö, Ljusdal, Östersund, and Vilhelmina. The mite was
204 present on just one individual from a sample of 189 in Ljusdal 2011, but the low prevalence
205 and absence in 2012 sample led to this being characterized as an infected non-persistent
206 immigrant individual. The pattern of mite incidence mirrors that observed between 2000
207 and 2002 (Figure 1a Left hand panel).

208 2. *Is absence of the mite from the north associated with inability of the parasite to grow*
209 *and transmit on northern ladybirds?*

210 Northern ladybirds from Östersund and Ljusdal were successfully infected after mating with
211 infectious partners derived from Stockholm. Infection was acquired in 14 of 15 cases where
212 the recipient ladybird was from the north, compared to 15 of 16 control cases (recipient
213 control ladybird from Stockholm) (Table 1a). Binomial GLM revealed no interaction between
214 location of recipient beetle and sex of recipient beetle. The model with the interaction term
215 removed showed no evidence for an effect of either location of origin of the recipient
216 beetle, nor sex of recipient ladybird, on the chance of acquiring infection (Table S2a).

217 We then examined the ability of the mite infection to develop on the northern (novel) hosts.
218 Eleven ladybirds out of 14 became infectious with the mite by day 17, two hosts died with a
219 live mite infection, and one host recovered (mite infection lost). This compared with 15
220 control recipients, where 10 hosts became infectious, five died with a live mite infection,
221 and no host recovered (Table 1b). Analysis indicated no evidence to reject the null
222 hypothesis of no impact of ladybird population source on the chance of recovery following
223 initial infection (Table S2b; interaction term dropped as non-significant). Data on latent
224 period was not collected on a daily basis, preventing formal analysis. However, the data do
225 not provide obvious signs of a difference in latent period between Stockholm and
226 Östersund/Ljusdal hosts (Table 1c).

227 Finally, we checked if mites can be transmitted onward from northern ladybird hosts. We
228 observed onward transmission from four of five experimental matings to uninfected
229 northern ladybirds, and from six of six control matings (Stockholm source and recipient)
230 (Table 1d). There was no evidence that origin of recipient beetle affected transmission of
231 infection (Table S2c).

232 *3. Phenology of the host: sexual contact between generations and general observations on* 233 *the timing of reproduction*

234 Temporal sampling in Stockholm: Overwintered and new cohort adult beetles in Stockholm
235 co-occurred for a period of more than a month in both 2010 (Figure 2, Suburban locations)
236 and 2011 (Figure 3, City centre locations). Mite infection on the new cohort beetles were
237 first observed about three weeks after the start of emergence of the cohort in both
238 populations, compatible with onset of reproductive activity (ladybird maturation takes 10
239 days under optimal food conditions). Mating activity of new cohort beetles was directly
240 observed in 2011, and commenced at week 6, the point at which mite infections were first
241 observed on the new cohort.

242 Notwithstanding the presence of overlap of cohorts in both years and in all locations within
243 Stockholm, there was obvious spatial and temporal heterogeneity in the pattern of overlap.
244 In 2010, reproduction commenced early in the suburban locations, and new generation
245 beetles were evident by the end of June. In contrast, collections from the city centre
246 locations in this year showed delayed recruitment, with no new generation beetles
247 observed until the start of August, and a possibility that the small number observed were
248 immigrants from other locations. In 2011 this pattern was reversed. Recruitment into the
249 city centre locations commenced from 15st June, with no recruitment observed in suburban
250 populations before 7th July.

251 Analysis of overlap between cohorts in mite free/mite present populations: Observations in
252 2012 were made more widely across Sweden, and focussed on the state of the population in
253 August, by which time old cohort beetles were expected to be rare or absent (Table 2).
254 Ladybirds collected in early August 2012 from the southern areas where the mite was
255 naturally present (Gävle and Stockholm) comprised both new and old generation adult

256 ladybirds, with mite infection established on the new cohort. Between 4% and 16% of new
257 cohort female beetles in these locations were fertile, indicating sexual contact had
258 commenced. In these populations, the emergence of the new generation was nearly
259 complete, with very few larvae/pupae remaining on trees.

260 In contrast, adult *Adalia* collected from areas where the mite was not present (Östersund
261 and Ljusdal north from 61°, and southern Nässjö) were solely of the new generation, with no
262 overwintered ladybirds still alive (Table 2). The emergence of the new cohort was ongoing,
263 as established from presence of 3rd/4th instar larvae and pupae on the trees. None of the
264 females collected in the two northern populations (Ljusdal and Östersund) were fertile.
265 Restoration of fertility following crossing in the laboratory indicated that the females in
266 these populations had not entered reproductive diapause, allowing us to be certain that lack
267 of fertility was associated with lack of previous mating activity (data not shown). Thus, in
268 this pair of populations, all females were virgin, there were no old-cohort individuals to
269 mate with, and reproductive contact with the overwintered cohort can be excluded. In
270 contrast to the northern populations, 12% of new-cohort beetles in Nässjö were fertile. It is
271 not possible to determine whether mated females in Nässjö had sexual contact with old
272 generation males that had since died out, or had mated with new cohort males.

273 General phenological information on the timing of ladybird reproduction: Observational
274 data from 2011 can be used to infer the timing of reproduction in the different populations.
275 Oviposition can be directly estimated from presence of eggs on trees, and also derived from
276 working back from observations of pupae on trees, with a guide that development to
277 pupation takes c. 21 days at 20°C. These data indicate an association between mite
278 presence and early oviposition by the overwintered cohort, with reproduction in mite-free
279 populations delayed compared to those where the mite was present (Table 3). This delay is
280 most pronounced in the northern mite-free populations.

281

282

283 Discussion

284 The impact of phenological variation on the dynamics of interactions between species is
285 becoming more appreciated. For parasite-host interactions, the targeting by the parasite of
286 particular host life history stages makes the dynamics of the system sensitive to shifts in the
287 phenology of one or both parties. For brood parasitic cuckoos, for instance, timing of cuckoo
288 reproductive activity compared to that of the host is vital: the interaction requires
289 coincident egg laying of cuckoo/host (Moller et al, 2011). We can hypothesize two further
290 factors that would increase sensitivity of host-parasite interactions to phenological shifts.
291 First, there is the level of host specificity of the parasite/pathogen. Specialization of a
292 parasite to particular host species would remove the possibility of alternate (or novel) hosts
293 maintaining the parasite in the face of phenological change, as observed in cuckoos. Second,
294 there is the mode of transmission. Pathogens that are directly transmitted on host contact
295 are likely to be more sensitive to phenological changes than those where there is infectivity
296 associated with propagules persisting in the environment, because direct transmission
297 narrows the 'window of opportunity' for transmission.

298 STIs meet these three criteria, being limited to sexually active adults, transmitted only
299 amongst members of one host species, and directly transmitted through host-host contact.
300 Our hypothesis was that an STI (a mite) could be debarred from being maintained on their
301 host (ladybird) simply through altered phenology of the host, as 'generation gaps' in which
302 adults of one cohort did not mate with adults of another would create a 'hard stop' to mite
303 persistence. We first established that patterns of mite presence/absence are stable over
304 time. Our observations of mite incidence in 2011 and 2012 are precisely congruent with
305 previous records from 2000-2002 (Webberley et al 2006b), with Swedish populations north
306 of 61° N latitude being uninfected with mites, and those south of this latitude being
307 infected, with one exception (Nässjö). Thus, we can infer a persistent biological/ecological
308 basis to the presence/absence of the mite. Our laboratory experiments indicate differential
309 ability of the mite to infect and develop on ladybirds does not explain variation in mite
310 presence. Ladybirds from northern populations were competent to acquire mites, develop
311 mite infections, and then transmit larval mites onward. Indeed, measures of parasite
312 performance on the host, such as latent period and per-contact transmission rates, revealed
313 no effect of host origin on the performance of the mite ectoparasite. Therefore, the factors

314 that could explain mite absence in the northern habitats lie in transmission biology
315 associated with host-host contacts.

316 Knell & Webberley (2004) suggest lack of sexual contact between cohorts as a potential
317 'hard stop' to STI persistence. In its most simple sense, an infection that can only exist on
318 adults, and not in the environment, can only persist if there are always adults present.
319 'Generation gaps' may be associated with life in temperate environments where winter
320 creates defined cohorts of insects that may not overlap, and may also select for diapause in
321 the egg stage. In addition, defined cohorts of insects may exist in tropical climates through
322 the process of generation cycles (Knell 1998), and may also be associated with aestivation in
323 regions with pronounced hot summers. Our observations provide strong evidence for that
324 phenology can create a 'hard stop' to mite transmission within the two northern
325 populations, with no sexual contact between the generations. Where dispersal is limited,
326 only occasional 'hard' stops are required to maintain a condition where mites are not
327 present.

328 Our phenological observations suggest that two phenomena contribute to the lack of sexual
329 contact between old and new generation beetles in northern populations. First, as expected
330 from latitude, host reproduction commences earlier in the mite-present populations, such
331 that the new generation emerges earlier. However, a second contributing factor was that
332 old generation beetles died off more rapidly in the northern populations despite their later
333 emergence from overwintering. There are no overwintered beetles in Östersund or Ljusdal
334 (61°50'N 16°05'E) in our early August samples, and in the 'edge of range' population from
335 Gävle, a single overwintered beetle was found at this time. We presume this rapid mortality
336 is associated with increased stress of a longer colder winter in northern latitudes. These two
337 forces combine to separate adult beetles from the overwintered and new cohort in time,
338 and prevent STI maintenance.

339 Phenological evidence from the 'mite present' populations was also consistent with
340 presence of sexual contact between generations. Temporal data from the Stockholm
341 population clearly indicated substantial overlap between cohorts in two field seasons. It is
342 notable that there is, nevertheless, spatial heterogeneity in overlap within Stockholm in
343 both years. In 2010, cohort overlap was pronounced in the suburban sites but nearly absent

344 in the inner city samples, and in 2011, pronounced in city centre sites but more weakly
345 present in the suburbs. These locations are within 12 km of each other, and indicate that
346 spatial variation in host phenology represents an important buffer against generation gaps.
347 To these data is added information from August 2012 collections. In these, both the
348 Stockholm and Gävle populations contained a mix of overwintered and new cohort
349 individuals during the August 2012 collections, and some new cohort females had mated.
350 Thus, sexual contact between new and old cohort individuals is highly likely to have
351 occurred in this year as well.

352 The Nässjö (57°39'N, 14°41'E) population, which is located south of Stockholm, presents a
353 more ambiguous case. This city lies at 375 metres above sea level in Småland, the highlands
354 of southern Sweden. Like the northern mite-absent populations, the overwintered cohort
355 had died off by August 2012. However, in contrast to these populations, recruitment of the
356 new cohort begins earlier in the year (equivalent to the northernmost mite-present
357 population of Gävle, but delayed compared to Malmö and Stockholm). It is likely that Nässjö
358 is on the margins of being able to retain mite infection, but that transfer between cohorts is
359 impossible in a fraction of years, such that the mite is generally absent.

360 In summary, the incidence of STIs in *A. bipunctata* is associated with the presence of
361 reproductive continuity between generations. More widely, we would expect increased
362 phenological sensitivity for parasites like STIs that are host-species specific, host life stage
363 specific, and have no (or limited) environmental survival. For insect STIs, we predict
364 incidence will decline towards polar regions, as cohort overlap is less likely. It is notable
365 that, for instance in Europe, many species show an obligate diapause in the north, such that
366 they will not engage in sexual activity until emergence from overwintering. We would
367 predict that for ladybird species such as *Coccinella septempunctata* (Phoofolo and Obrycki
368 2000), where there is a clinal gradient in diapause requirement, the incidence of the sexually
369 transmitted mite *C. macfarlanei* would correspond to the geographical region where there is
370 no diapause requirement.

371 Acknowledgements. We wish to thank Andy Fenton and two anonymous referees for helpful
372 comments on the manuscript, Karl Gotthard for assistance during field work in Sweden, and

373 the NERC (grant NE/G003246/1 to GH, DA, RK, MB) and a University of Liverpool
374 studentship (DP) for funding.

375 References

376

- 377 Godfray, H. C. J. et al. 1994. The population-dynamic consequences of phenological asynchrony
378 between parasitoids and their hosts. - J. Anim. Ecol. 63: 1-10.
- 379 Hurst, G. D. D et al. 1995. Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*. -
380 Ecol. Entomol. 20: 230-236.
- 381 Johansson, J. et al. 2015. Phenological change and ecological interactions: an introduction. - Oikos
382 124: 1-3.
- 383 Knell, R. J. 1998. Generation cycles. - Trends Ecol. Evol. 13: 186-190.
- 384 Knell, R. J. and Webberley, K. M. 2004. Sexually transmitted diseases of insects: distribution, ecology,
385 evolution and host behaviour. - Biol. Rev. 79: 557-581.
- 386 Majerus, M. E. N. 1994. Ladybirds. - Harper-Collins.
- 387 Moller, A. P. et al. 2011. Rapid change in host use of the common cuckoo *Cuculus canorus* linked to
388 climate change. - Proc. R. Soc. Lond. B 278: 733-738.
- 389 Paull, S. H. and Johnson, P. T. J. 2014. Experimental warming drives a seasonal shift in the timing of
390 host-parasite dynamics with consequences for disease risk. - Ecol. Lett. 17: 445-453.
- 391 Phoofolo, M. W. and Obrycki, J. J. 2000. Demographic analysis of reproduction in Nearctic and
392 Palearctic populations of *Coccinella septempunctata* and *Propylea quatuordecimpunctata*. -
393 BioControl 45: 25-43.
- 394 Ryder, J. J. et al. 2013. Spatial variation in food supply, mating behavior, and sexually transmitted
395 disease epidemics. - Behav. Ecol. 24: 723-729.
- 396 Ryder, J. J. et al. 2014. Disease Epidemiology in Arthropods Is Altered by the Presence of
397 Nonprotective Symbionts. - Am. Nat. 183: E89-E104.
- 398 Seeman, O. D. and Nahrung, H. F. 2004. Female biased parasitism and the importance of host
399 generation overlap in a sexually transmitted parasite of beetles. - J. Parasitol. 90: 114-118.
- 400 Thomson, L. J. et al. 2010. Predicting the effects of climate change on natural enemies of agricultural
401 pests. - Biol. Cont. 52: 296-306.
- 402 Webberley, K. M. et al. 2006a. Sexually Transmitted Disease Epidemics in a Natural Insect
403 population. - J. Anim. Ecol. 75: 33-43.
- 404 Webberley, K. M. et al. 2006 b. Spatial variation in the incidence of a sexually transmitted parasite of
405 the ladybird beetle *Adalia bipunctata* (Coleoptera : Coccinellidae). - Eur. J. Ent. 103: 793-797.
- 406

407 Table 1: Ability of *A. bipunctata* from the northern populations of Östersund/Ljusdal to
 408 acquire, retain and transmit mites in comparison to beetles from Stockholm.

409 a) The proportion of ladybirds that acquired mites after mating with a mite-infected
 410 partner, partitioned by sex and origin of recipient

Origin of recipient beetle	Sex of recipient beetle	<i>P</i> (infected through mating) (<i>N</i>)
Stockholm	Male	0.89 (9)
Stockholm	Female	1.0 (7)
Ljusdal/Östersund	Male	0.86 (7)
Ljusdal/Östersund	Female	1.0 (8)

411

412 b) The fate of mite infection, partitioned by location and sex of host.

Origin of recipient beetle	Sex of recipient beetle	Number infected	Fate of infection		
			Host recovered	Host died before becoming infectious	Host became infectious
Stockholm	Male	8	0	2	6
Stockholm	Female	7	0	3	4
Ljusdal/Östersund	Male	6	1	0	5
Ljusdal/Östersund	Female	8	0	2	6

413

414 c) Estimated latent period of mite infection, partitioned by location and sex of host.

Origin of recipient beetle	Sex of recipient beetle	Number	Latent period		
			≤14 days	15-17 days	>17 days
Stockholm	Male	6	5	1	0
Stockholm	Female	4	2	2	0
Ljusdal/Östersund	Male	5	3	1	1
Ljusdal/Östersund	Female	6	4	2	0

415

416

417

418

419 d) The proportion of ladybirds that transferred mites onwards during copulation with an
420 uninfected partner, partitioned by origin and sex of host.

Origin of recipient beetle	Sex of recipient beetle	<i>P</i> (ladybirds infected through mating) (<i>N</i>)
Stockholm	Male	1.0 (4)
Stockholm	Female	1.0 (2)
Östersund	Male	0.5 (2)
Östersund	Female	1.0 (3)

421

422

423

424 Table 2: Presence of overwintered and new cohort adult *A. bipunctata* beetles in August
 425 2012 at five locations in Sweden, alongside data on fertility of new cohort females collected.
 426 Other life stages: L3= 3rd instar larvae L4=4th instar larvae P=Pupae. Locations are ordered
 427 from north to south.

428

Place	Date	Mite present	# old generation adults	# new generation adults	# fertile new generation females	Other life stages present
Östersund	08/08	No	0	99	0/25	L4, P
Ljusdal	09/08	No	0	92	0/25	L3, L4, P
Gävle	09/08	Yes	1	56	1/25	None
Stockholm	11/08	Yes	112	57	4/25	P
Nässjö	10/08	No	0	62	3/25	L3, L4, P

429

430 Table 3: Inferred timing of reproduction in different Swedish populations in 2011 from
 431 observations of egg, larval and pupal presence during collections. Data are ordered by
 432 earliest reproduction first.

433

Population	Mite status	Longitude Latitude	Estimated time of first oviposition by overwintered cohort	Evidence
Stockholm	Present	59°19'N 18°4'E	Before 21/05	Abundant eggs observed 21/05
Malmö	Present	55°35'N 13°02'E	Around 20/05	Presence of a few pupae in sample 11/06
Gävle	Present	60°40'N 17°10'E	Between 04/06 and 19/06	No eggs present on 04/06; abundant pupae on 08/07
Nässjö	Absent	57°39'N 14°41'E	Between 12/06 and 19/06	No eggs present on 12/06; abundant pupae on 09/07
Ljusdal	Absent	61°50'N 16°05'E	Between 17/06 and early July	No eggs on 17/06; pupae present on 28/07
Vilhelmina	Absent	64°37'N 16°39'E	After 03/07	No eggs or larvae on 03/07
Östersund	Absent	63°11'N 14°40'E	After 04/07	No eggs or larvae on 04/07

434

435

436 Figure 1. The incidence of the mite at various locations in Sweden/Norway; x marks location
437 of collection. Symbols below location names indicate years of ladybird collection (left hand
438 block: triangle = 2000, square = 2001, + = 2002; right hand block: Circle =2011, Diamond =
439 2012) and presence (blue fill) or absence (orange fill) of mites in the sample. Data for 2000-
440 2002 taken from Webberley et al., 2006b.

441

442 Figure 2: Phenology of old and new cohort beetles at city centre and suburban locations in
443 Stockholm, Sweden, in Spring/Summer 2010. Bars represent numbers of overwintered
444 (green) and new cohort (blue) adult beetles collected during census. Lines represent
445 prevalence of *C. hippodamiae* in old (orange line) and new cohort (red) adult beetles, with
446 binomial confidence interval error bars. x-axis is time elapsed in weeks from start of
447 observations (17th May).

448

449 Figure 3: Phenology of old and new cohort beetles at four locations in Stockholm, Sweden,
450 in Spring/Summer 2011. Bars represent numbers of overwintered (green) and new cohort
451 (blue) adult beetles. Lines represent prevalence of *C. hippodamiae* in old (orange line) and
452 new cohort (red) beetles, with binomial confidence interval error bars. X-axis is time elapsed
453 in weeks from start of observations (21st May).







