

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

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**(Abstract)**

Changes in the timing of life history events within the year alter the degree to which the activity patterns of different species coincide, making the dynamics of interspecific interactions sensitive to the phenology of the interacting parties. For parasites, the availability of suitable hosts to infect represents a crucial determinant of dynamics, and changes in the host (and parasite) phenology may thus alter disease epidemiology and the conditions for disease maintenance. We tested the hypothesis that the incidence of a sexually transmitted mite infection, *Coccipolipus hippodamiae*, in *Adalia bipunctata* ladybird beetles in Sweden was determined by host phenology, namely presence/absence of sexual contact between cohorts of the host. We observed that the pattern of mite presence/absence across Swedish *A. bipunctata* populations was highly reproducible between years, implying a persistent biological/ecological basis underlying the incidence. Further, ladybirds from populations where the mite was absent were able to acquire mites during copulation, develop a mite infection, and transmit infection onward, indicating an ecological (rather than biological) driver of mite incidence. Observations of ladybird phenology in natural populations provided evidence of sexual contact between overwintered and new cohort adults in populations where the mite was present. In contrast, new cohort ladybirds in the two northern Swedish populations where the mite was not present had not had sexual contact with the overwintered generation, creating a ‘hard stop’ to mite transmission. We conclude that variation in host phenology may be an important driver of the incidence of sexually transmitted infections (STIs) by determining the presence/absence of sexual contact between generations. More generally, we hypothesize that sensitivity to variation in host phenology will be highest for parasites like STIs that infect one host species, one host life stage and are directly transmitted on contact between host individuals.

## Introduction

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

Recent studies have suggested a potential role of phenological variation in driving the incidence of sexually transmitted infections (STIs) of invertebrates (Hurst et al. 1995, Seeman and Nahrung 2004). STIs are unusual in that they are confined to adults, and are transmitted directly during host copulation. As Knell & Webberley (2004) argue, infections that are solely transmitted during host copulation can only be maintained where cohorts of adults overlap and there is inter-generational mating that allows the parasite to transmit to the new cohort. A ‘pure’ STI would disappear from a host population where there was a ‘generation gap’: periods where no adult individuals are present, or are present but have not commenced mating activity. For invertebrates in temperate environments, generation gaps may be a product of either seasonal forcing into cohorts or obligate diapause requirement. Gaps may also arise from ‘generation cycles’ in tropical species (Godfray & Hassell, 1989; Knell 1998). Despite the logic of this argument, no empirical study to date has directly investigated the importance of host phenology as a driver of STI/host dynamics (Knell and Webberley 2004).

In this study we assess whether phenological variation in the host drives the presence/absence of an STI. The *Adalia bipunctata/Coccipolipus hippodamiae* two spot ladybird/mite interaction provides an excellent test system for this hypotheses, as the basic biology of the interaction and transmission processes are well established (Hurst et al. 1995), there is a good working knowledge of mite epidemiology within a cohort (Webberley et al 2006a, Ryder et al. 2013, Ryder et al. 2014), and geographical

variation in the presence of the mite STI that has not been explained (Webberley et al. 2006b).

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

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

## Methods

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

Overwintered *A. bipunctata* were collected in 2011 and 2012 from Swedish urban habitats (city centres and suburbs) of Malmö, Nässjö, Stockholm, Gävle, Ljusdal,

Östersund and Vilhelmina, mirroring locations previously sampled by Webberley et al. (2006b) (Fig. 1). Ladybirds were collected from trees into a beating tray and from bushes by hand collection. Collected individuals were transferred to 1.5 ml Eppendorf tubes. Ladybirds that were caught as single individuals were confined singly, to prevent any contagion during storage. If a mating pair landed on the beating tray, ladybirds were not separated but transferred together into the tube.

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

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

We tested the ability of ladybirds from populations that did not carry mites naturally to become infected with larval mites, develop infection and transmit the mite infection onward. To this end, *A. bipunctata* were collected using a beating tray in Östersund and Ljusdal (where the mite is absent) and in Stockholm (where the mite is naturally present) in early August 2012. These ladybirds were then maintained in an incubator at 20°C with a light regime of 20L:4D (20 hours of light and 4 hours of night), and fed aphids daily.

Thirteen uninfected ladybirds from Östersund (mites naturally absent), six uninfected ladybirds from Ljusdal (mites naturally absent) and 19 uninfected ladybirds from Stockholm (mites naturally present, control) were isolated individually in Petri dishes and paired with a mite-infected partner (from Stockholm) and mating observed. The next day ladybirds were separated from their infectious partner and then checked for the presence of larval mites (successful initial transmission). Mite persistence and disease

latent period (the time from initial infection to infectious larval forms being produced) were then measured. To this end, recipient ladybirds were checked for the progress of mite infection on the seventh, fourteenth and seventeenth day post infection, and thereafter daily. Where infection developed, the ability of the host to transmit mites onwards was tested. One week after the ladybirds above were first scored as carrying larval mites (the infectious stage), they were paired with an uninfected partner from the same population. Mating was observed as before, and the recipient checked for larval mite presence the next day.

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

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

Temporal sampling in Stockholm: We recorded the presence of overwintered and new cohort beetles, and prevalence of mite infection in Stockholm (mite present population) in weekly collections over the periods 17<sup>th</sup> May 2010-15<sup>th</sup> August 2010, and 21<sup>st</sup> May-27 July 2011. Beetles were collected by beating from lime trees in city centre and suburban habitats, with aim of collecting 150/100 beetles (2010/2011 respectively), or if this number of beetles could not be obtained, that which could be found in one hour. Individuals were also noted as mating/single on collection. Collected beetles were sexed, scored for mite presence, and scored as to cohort through elytral colour, with individuals belonging to an overwintered generation being deep red/black, in contrast to the orange/red of adults from the newly emerged generation (Majerus 1994).

Analysis of overlap between cohorts in mite-free/mite-present populations. From the Stockholm data, it was clear that July and August represents the period when overwintered and new generation adult ladybirds may be present together, a necessary condition for transfer of the mite between generations. A break in the transmission cycle would occur when new generation ladybirds emerged after the death of overwintered

cohort. This would be evidenced by the presence of virgin new cohort beetles in August with no overwintered beetles present.

The co-occurrence of new and overwintered cohort adult ladybirds, and the matedness of female new cohort ladybirds, was therefore examined in five different populations in August 2012 spanning the region where the mite showed presence/absence differentiation: Östersund, Ljusdal (northern, both mite-absent), Gävle, Stockholm (both mite-present) and Nässjö (southern, mite-absent). Adult beetles were collected by beating lime tree branches using a beating tray and by hand, and transported to the laboratory where they were scored for sex, mite presence and for cohort. Alongside the collection of adult beetles, notes were made on the presence of other life history stages in the population, as an indicator of the timing of new cohort emergence.

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

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

## Results

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



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

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

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

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

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

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



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

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

Analysis of overlap between cohorts in mite-free/mite-present populations:

Observations in 2012 were made more widely across Sweden, and focussed on the state of the population in August, by which time old cohort *A. bipunctata* were expected to be rare or absent (Table 2). Ladybirds collected in early August 2012 from the southern areas where the mite was naturally present (Gävle and Stockholm) comprised both new and old generation adult *A. bipunctata*, with mite infection established on the new cohort. Between 4% and 16% of new cohort female *A. bipunctata* in these locations were fertile, indicating sexual contact had commenced. In these populations, the emergence of the new generation was nearly complete, with very few larvae/pupae remaining on trees.

In contrast, adult *Adalia* collected from areas where the mite was not present (Östersund and Ljusdal north from 61°, and southern Nässjö) were solely of the new generation, with no overwintered ladybirds still alive (Table 2). The emergence of the new cohort was ongoing, as established from presence of 3<sup>rd</sup>/4<sup>th</sup> instar larvae and pupae on the trees. None of the females collected in the two northern populations (Ljusdal and Östersund) were fertile. Restoration of fertility following crossing in the laboratory indicated that

the females in these populations had not entered reproductive diapause, allowing us to be certain that lack of fertility was associated with lack of previous mating activity (data not shown). Thus, in this pair of populations, all females were virgin, there were no old-cohort individuals to mate with, and reproductive contact with the overwintered cohort can be excluded. In contrast to the northern populations, 12% of new-cohort beetles in Nässjö were fertile. It is not possible to determine whether mated females in Nässjö had sexual contact with old generation males that had since died out, or had mated with new cohort males.

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

## **Discussion**

The impact of phenological variation on the dynamics of interactions between species is becoming more appreciated. In general, the sensitivity of parasite-host interactions to phenological shifts will be greatest where there is a narrow ‘window of opportunity’ for a parasite or pathogen to establish new infections. It is thus the case that parasites which target particular host life history stages will be more sensitive to phenological shifts than those with a broad acceptance of host life history stages. For brood parasitic cuckoos, for instance, phenological sensitivity derives from the need for coincident egg-laying of the cuckoo and its host (Møller et al, 2011). We can hypothesize two further aspects of parasite-host interactions that would narrow the ‘window of opportunity’ for infection and thus increase sensitivity to changes in phenology. First, there is the host range of the parasite/pathogen. Specialization of a parasite to particular host species narrows the time period over which infection may be established by removing the possibility of alternate (or novel) hosts maintaining the parasite. Second, the mode of transmission of the pathogen may alter the breadth of the ‘window’. Pathogens/parasites with direct transmission between host individuals have a shorter period over which infection can be established than pathogens/parasites which infect via propagules which

have environmental persistence. Thus, direct transmission is likely to lead to increased sensitivity to shifts in host/parasite phenology.

STIs have several characteristics that would be likely to narrow the ‘window of opportunity’ for infection and hence lead them to have infection processes that are phenologically sensitive. By their nature, STIs are limited to sexually active adults, transmitted only amongst members of one host species, and directly transmitted through host-host contact. Our hypothesis was that persistence of an STI (infection by a mite) on their host (ladybird) could be prevented simply through altered phenology of the host. We reasoned that ‘generation gaps’, in which adults of one cohort did not mate with adults of the next, would create a ‘hard stop’ to mite persistence as there are no alternate hosts to act as pathogen/parasite reservoirs, and no environmental persistence of infectious propagules.

Testing this hypothesis, we first established that patterns of mite presence/absence were stable over time. Our observations of mite incidence in 2011 and 2012 are precisely congruent with previous records from 2000-2002 (Webberley et al 2006b), with Swedish populations north of 61° N latitude being uninfected with mites, and those south of this latitude being infected, with one exception (Nässjö). Thus, we can infer a persistent biological/ecological basis to the presence/absence of the mite. Our laboratory experiments then excluded a biological basis to mite absence. Ladybirds from northern populations (where the mite was absent) were competent to acquire mites, develop mite infections, and then transmit larval mites onward. Indeed, measures of parasite performance on the host, such as latent period and per-contact transmission rates, revealed no effect of host origin on the performance of the mite ectoparasite. Therefore, the factors that explain the persistent pattern of mite presence/absence lie in ecological rather than biological differences between populations.

Knell & Webberley (2004) suggest lack of sexual contact between cohorts as a potential ‘hard stop’ to STI persistence. In its most simple sense, an infection that can only exist on adults, and not in the environment, can only persist if there are always adults present. ‘Generation gaps’ may be associated with life in temperate environments where winter creates defined cohorts of insects that may not overlap. In addition, defined cohorts of insects may exist in tropical climates through the process of generation cycles (Knell 1998), and may also be associated with aestivation in regions with pronounced hot summers. Our observations provide strong evidence that phenology can create such a

‘hard stop’ to mite transmission within the two northern populations, with no sexual contact between the generations. Where dispersal is limited, only occasional ‘hard’ stops are required to maintain a condition where mites are not present.

Our phenological observations suggest that two phenomena contribute to the lack of sexual contact between old and new generation beetles in northern, compared with southern populations. First, host reproduction commences earlier in the southern, generally mite-present, populations, such that the new cohort of adults emerges earlier in the year. Second, old generation beetles die off more rapidly in the northern populations despite their later emergence from overwintering. There were no overwintered beetles in Östersund or Ljusdal (61°50’N 16°05’E) in our early August samples, and in the ‘edge of range’ population from Gävle, a single overwintered beetle was found at this time. We presume this rapid mortality of beetles in summer is associated with increased stress during a longer colder winter in northern latitudes. These two forces combine to separate the overwintered from new cohort adult beetles, and prevent STI persistence.

Phenological evidence from the ‘mite-present’ populations was also consistent with presence of sexual contact between generations. Observed co-occurrence of beetle generations in the Stockholm population clearly indicated substantial overlap between overwintering and new cohorts in each of two field seasons. It is notable that there is, nevertheless, spatial heterogeneity in overlap within Stockholm in both years. In 2010, cohort overlap was pronounced in the suburban sites but nearly absent in the inner city samples, and in 2011, pronounced in city centre sites but more weakly present in the suburbs. These locations are within 12 km of each other, and indicate that spatial variation in host phenology represents an important buffer against generation gaps. To these data is added information from August 2012 collections. In these, both the Stockholm and Gävle populations contained a mix of overwintered and new cohort individuals during the August 2012 collections, and some new cohort females had mated. Thus, sexual contact between new and old cohort individuals is highly likely to have occurred in this year as well.

The Nässjö (57°39’N, 14°41’E) population, which is located south of Stockholm, presents a more ambiguous case. This city lies at 375 metres above sea level in Småland, the highlands of southern Sweden. Like the northern mite-absent populations, the overwintered cohort had died off by August 2012. However, in contrast to these

populations, recruitment of the new cohort begins earlier in the year (equivalent to the northernmost mite-present population of Gävle, but delayed compared to Malmö and Stockholm). It is likely that Nässjö is on the margins of being able to retain mite infection, but that transfer between cohorts is impossible in a fraction of years, such that the mite is generally absent.

In summary, the incidence of STIs in *A. bipunctata* is associated with the presence of reproductive continuity between generations. More widely, we would expect the incidence of parasite infections, including STIs, to be particularly phenologically sensitive when they are host-species specific, host life stage specific, and have no (or limited) environmental survival. For insect STIs, we predict incidence will decline towards polar regions, as cohort overlap is less likely. It is notable that, for instance in Europe, many species show an obligate diapause in the north, preventing sexual activity until emergence from overwintering. We would predict that for ladybird species such as *Coccinella septempunctata* (Phoofolo and Obrycki 2000), where there is a clinal gradient in diapause requirement, the incidence of the sexually transmitted mite *C. macfarleni* would correspond to the geographical region where there is no diapause requirement.

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## Table Legends

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

- a) The proportion of ladybirds that acquired mites after mating with a mite-infected 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> )
<b>Stockholm</b>	Male	0.89 (9)
<b>Stockholm</b>	Female	1.0 (7)
<b>Ljusdal/Östersund</b>	Male	0.86 (7)
<b>Ljusdal/Östersund</b>	Female	1.0 (8)

- 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
<b>Stockholm</b>	Male	8	0	2	6
<b>Stockholm</b>	Female	7	0	3	4
<b>Ljusdal/Östersund</b>	Male	6	1	0	5
<b>Ljusdal/Östersund</b>	Female	8	0	2	6

- 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
<b>Stockholm</b>	Male	6	5	1	0
<b>Stockholm</b>	Female	4	2	2	0
<b>Ljusdal/Östersund</b>	Male	5	3	1	1
<b>Ljusdal/Östersund</b>	Female	6	4	2	0

- d) The proportion of ladybirds that transferred mites onwards during copulation with an 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> )
<b>Stockholm</b>	Male	1.0 (4)
<b>Stockholm</b>	Female	1.0 (2)
<b>Östersund</b>	Male	0.5 (2)
<b>Östersund</b>	Female	1.0 (3)

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

Place	Date	Mite present	# old generation adults	# new generation adults	fraction of new generation adults tested that were fertile	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

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

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

## Figure Legends

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

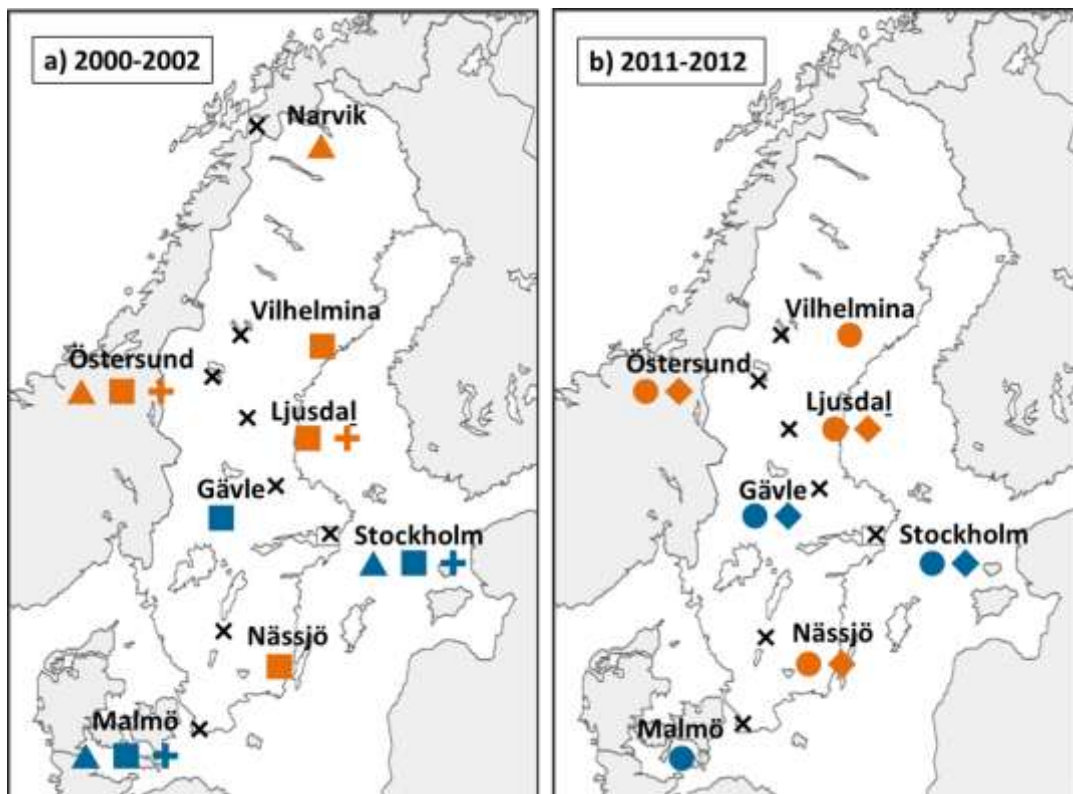


Figure 2: Phenology of old and new cohort beetles at city centre and suburban locations in Stockholm, Sweden, in Spring/Summer 2010. Bars represent numbers of overwintered (green) and new cohort (blue) adult beetles collected during census. Lines represent prevalence of *C. hippodamiae* in old (orange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

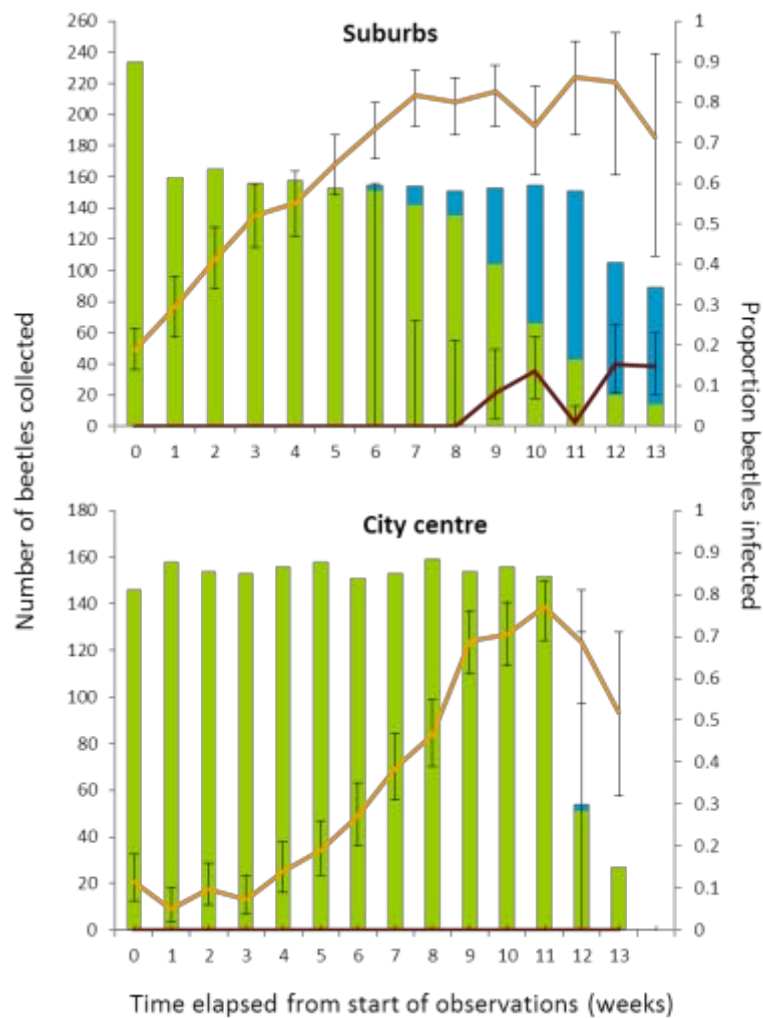


Figure 3: Phenology of old and new cohort beetles at four locations in Stockholm, Sweden, in Spring/Summer 2011. Bars represent numbers of overwintered (green) and new cohort (blue) adult beetles. Lines represent prevalence of *C. hippodamiae* in old (orange line) and new cohort (red line) beetles, with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

