

Population dynamics and sex ratio of a parasitoid altered by fungal-infected diet of host butterfly

Saskya van Nouhuys* and Anna-Liisa Laine

Metapopulation Research Group, Department of Biological and Environmental Sciences, University of Helsinki, PO Box 65, 00014 Helsinki, Finland

Variation of host quality affects population dynamics of parasitoids, even at the landscape scale. What causes host quality to vary and the subsequent mechanisms by which parasitoid population dynamics are affected can be complex. Here, we examine the indirect interaction of a plant pathogen with a parasitoid wasp. Under laboratory conditions, parasitoids from hosts fed fungus-infected plants weighed less than those from hosts fed uninfected plants, indicating that the fungus causes the hosts to be of poor quality. However, parasitoids reared from hosts fed fungal-infected diet also tended to be female, a characteristic associated with high host quality. The pathogen, herbivore and parasitoid persist regionally as metapopulations in a shared landscape in Åland, Finland. In an analysis of the metapopulation dynamics of the parasitoid over 6 years, the probability of colonization of a host population increased by more than twofold in patches occupied by the plant pathogen. While we cannot determine that the relationship is causal, a compelling explanation is that the plant pathogen facilitates the establishment by the parasitoid by increasing the fraction of female offspring. This is a novel mechanism of spatial multi-trophic level interactions.

Keywords: *Cotesia melitaearum*; host–parasite interaction; *Melitaea cinxia*; metapopulation; multi-trophic interaction; phytopathogen

1. INTRODUCTION

Population dynamics of insects are influenced by the interactions among species. One of the strongest of these interactions is between hosts and their parasitoids. The role of the host–parasitoid interaction in the population dynamics of both players can be mediated by the interactions with yet other species. An important contributor to host–parasitoid interactions is the substrate that the host feeds on (as reviewed by Bottrell *et al.* 1998; Price *et al.* 2004; Ode 2006). Host plants can affect parasitism of herbivores directly by attracting parasitoids (Vet & Dicke 1992) and indirectly through their effect on host accessibility or quality (Ode 2006). For instance, plant defensive chemistry and nutrient availability influence rate of parasitism, and offspring size, rate of development and survival (e.g. Barbosa *et al.* 1991; Turlings & Benrey 1998; Harvey *et al.* 2005; Rodriguez-Saona *et al.* 2005; Harvey *et al.* 2007). Furthermore, because resource needs and fitness values of male and female offspring differ (Charnov *et al.* 1981; Godfray 1994; Hardy 1998) and adult female parasitoids control the sex of their progeny, host plant quality can affect parasitoid sex ratio (Fox *et al.* 1990).

Phytopathogens influence the quality of plants as food for herbivores (Walters & Ayres 1981; Slansky & Wheeler 1992; Hatcher 1995; Moran & Schultz 1998; Biere *et al.* 2004; Stout *et al.* 2006). Qualitative changes following infection may lead to an increase in non-digestible matter and the induction of the host plant's resistance pathways,

leading to increased levels of secondary compounds and changes in water, carbohydrates and nitrogen content that can affect phytophagous insects (Mattson 1980; Hatcher 1995; Agrios 1997; Bushnell 2002; Cardoza *et al.* 2003; Biere & Honders 2006). Generally, pathogenic fungal infection on a shared host plant is considered to have negative effects on phytophagous insects (for reviews, see Hatcher 1995; Stout *et al.* 2006; for an exception, see Mondy & Corio-Costet 2004). Very little is known about the effects of fungal phytopathogens on trophic levels above insect herbivores, despite the great abundance and diversity of plant pathogens (Ingram & Robertson 1999). The few studies that have been conducted show high parasitism of herbivores feeding on fungal-infected plants, due to changes in plant structure (Biere *et al.* 2002) or composition of volatile emissions (Cardoza *et al.* 2003). Endophytic infection, though not detrimental to the host plant, also alters plant physiology. Endophytes have been found to have no effect above the herbivore trophic level (Urrutia *et al.* 2007), an effect that depends on fungal isolate (Bultman *et al.* 2003), or a clear negative effect on higher trophic levels (Omacini *et al.* 2001). While we know of no studies of the effect of pathogenic infection at the community level, Omacini *et al.* (2001) demonstrate that an endophytic infection played a central role in the composition of a multi-trophic level insect community.

In this study we demonstrate experimentally that the effects of phytopathogens can cascade through several trophic levels and alter the performance and sex ratio of a specialist parasitoid wasp. The study was carried out using a set of closely interacting species occupying a highly fragmented landscape in the Åland Islands in south

* Author for correspondence (sdv2@cornell.edu).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2007.1588> or via <http://journals.royalsociety.org>.

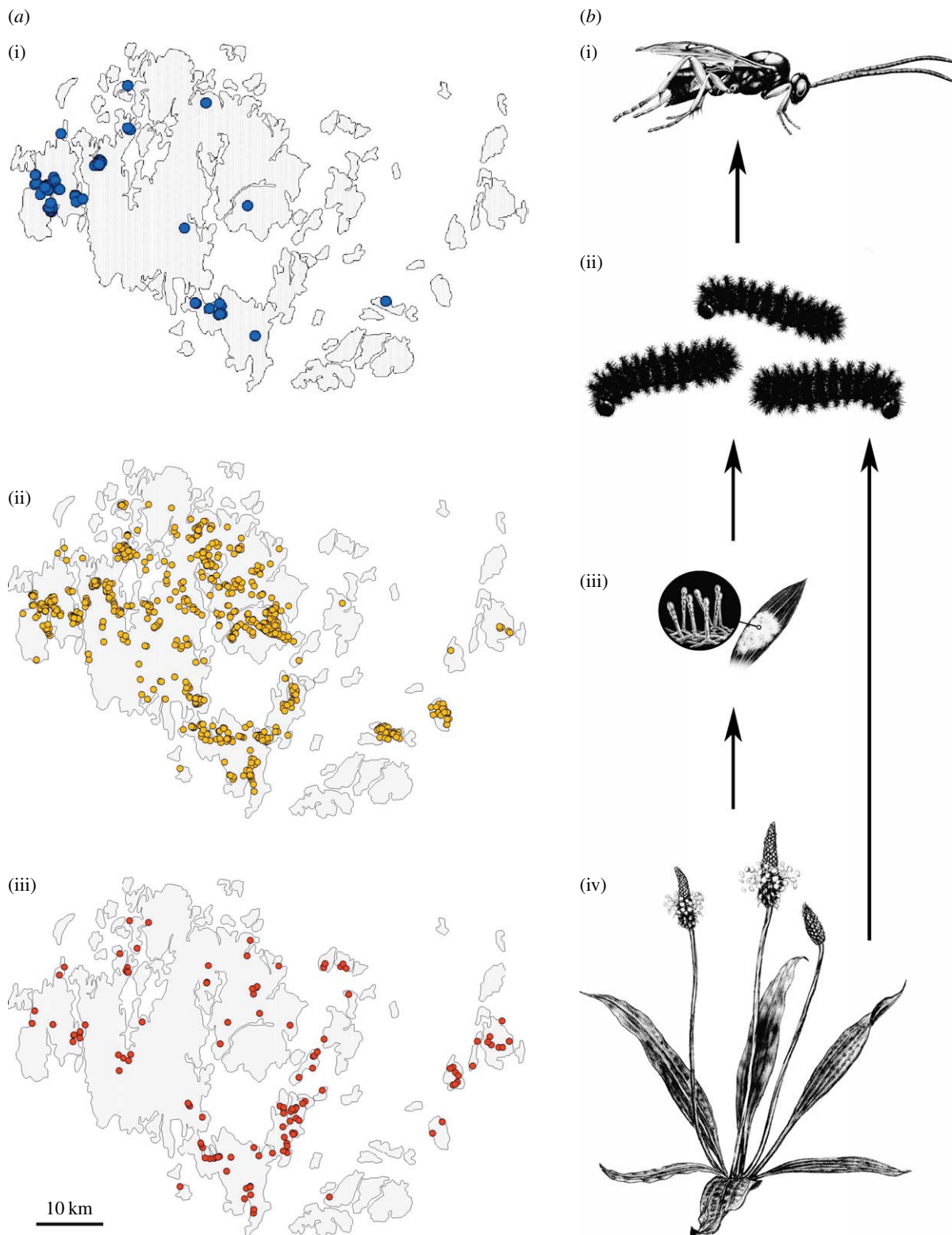


Figure 1. (a) Distribution maps of habitat patches occupied by (i) *C. melitaearum*, (ii) *M. cinxia* and (iii) *P. plantaginis* in the Åland Islands in 2005. (b) Trophic chain of the study species with (i) the parasitoid of *M. cinxia*, *C. melitaearum*, (ii) the herbivore *M. cinxia*, (iii) the powdery mildew *P. plantaginis* and (iv) the plant *P. lanceolata*. Drawings by Zdravko Kolev.

western Finland (figure 1). Both the specialist phytopathogen, *Podosphaera plantaginis* (Castagne, U. Braun & S. Takamatsu; Erysiphales) and the butterfly *Melitaea cinxia* (Lepidoptera: Nymphalidae) inhabit the host plant, *Plantago lanceolata* L. (Plantaginaceae). The phytopathogen exploits the nutrient resources of *P. lanceolata* to the extent that when infection coincides with other stress factors, it may induce host mortality

(Laine 2004a). Fungal-infected diet is also known to have negative effects on the development and survival of the larvae of *M. cinxia* (Laine 2004b). Here, we present effects of infection by *P. plantaginis* on both *M. cinxia* and *Cotesia melitaearum* (Hymenoptera: Braconidae), a specialist parasitoid of *M. cinxia*. Additionally, we analysed the interaction of the spatial and temporal dynamics of these species in their natural populations, in

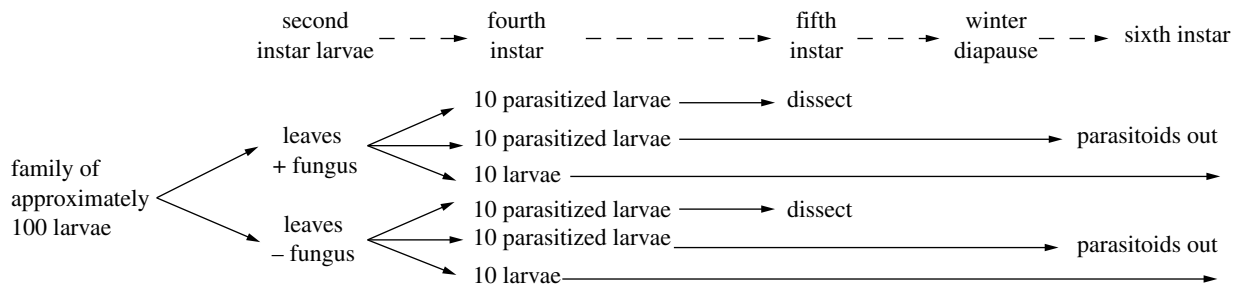


Figure 2. Schematic representation of the experimental design for one of the nine replicate families of *M. cinxia*.

an area of 50×70 km over 6 years. This analysis allows us to discern the potential roles of effects measured under laboratory conditions on a landscape and metapopulation scale.

2. MATERIAL AND METHODS

(a) Study system

(i) Host plant

The host plant *P. lanceolata* is a perennial herb with a cosmopolitan distribution, growing in poor soil and in open or disturbed habitat. It is considered an obligate outcrosser (Ross 1973), with seeds that drop to the ground close to the mother plant (Bos 1992; van Damme 1992) and frequent reproduction through clonal side rosettes (Mook *et al.* 1992). In the Åland Islands, it is typically found on dry rocky outcrops and grazed meadows distributed across a rural agricultural landscape.

(ii) Plant pathogen

Podospaera plantaginis is an obligate powdery mildew fungus in the order Erysiphales within the Ascomycota (Yarwood 1978). In Åland, it appears to be restricted to *P. lanceolata* (A.-L. Laine 2001, unpublished data). Infection is initiated as a spore germinates on the host surface. The fungus completes its entire life cycle on the surface of the host plant where it is visible as localized (non-systemic) white powdery lesions. The epidemics build up within host populations during summer, frequently resulting in a large proportion of the plants in a local population being infected (Ovaskainen & Laine 2006).

(iii) Butterfly host

The Glanville fritillary butterfly, *M. cinxia*, is a Eurasian butterfly that is extinct from or endangered in most of western and northern Europe. In Finland, it is found only in the Åland Islands, where it lays eggs in clusters on the host plants *P. lanceolata* and *Veronica spicata* L. (Plantaginaceae). The larvae live gregariously on the host plant in a silken web throughout their development, including a winter diapause (Kuussaari *et al.* 2004).

(iv) The parasitoid *C. melitaeorum*

Cotesia melitaeorum is an aggregate of cryptic parasitoid species associated with the checkerspot butterflies in the genera *Euphydryas* and *Melitaea* (Kankare & Shaw 2004). The species of *C. melitaeorum* agg. that uses *M. cinxia* has no other host species (Kankare *et al.* 2005). *Cotesia melitaeorum* is multivoltine, with two or three generations per host generation (year). A female lays up to 40 eggs in an *M. cinxia* larva, and the brood develops gregariously. The number of eggs laid depends to a large extent on the size of the host

larva when it is parasitized. During the parasitoid's last instar, it leaves the host to pupate nearby.

(v) The Åland metapopulation

The study system in the Åland Islands comprises approximately 4000 small meadows (less than 1 ha) containing *P. lanceolata* (Nieminen *et al.* 2004). The occurrences of all three species associated with *P. lanceolata* (the powdery mildew, butterfly and parasitoid) have been systematically followed in the meadow network since 2001, 1993 and 1997, respectively. The butterfly is censused annually by surveying suitable habitat patches for larval nests in the autumn. At the same time, *P. lanceolata* leaves in each habitat patch are inspected for powdery mildew, and the severity of infection is scored (Laine & Hanski 2006). The number of *M. cinxia* larvae in occupied patches is counted again each spring (Nieminen *et al.* 2004). At that time, each host nest is also searched for *C. melitaeorum* cocoons to assess the presence of the wasps in local butterfly populations and estimate the parasitoid population size (van Nouhuys & Hanski 2002).

All three species persist regionally as metapopulations. In a given year, *M. cinxia* occupies roughly 15–20% of its available host populations (Nieminen *et al.* 2004), while occupancy levels for *P. plantaginis* and *C. melitaeorum* have remained lower at approximately 5 and 5–15%, respectively, with considerable annual fluctuations (figure 1; van Nouhuys & Hanski 2002; Laine & Hanski 2006).

(b) The experimental design

The effects of fungus-infected diet on the host butterfly and the parasitoid were studied in a laboratory experiment in which host larvae were fed either healthy or fungus-infected leaves taken from natural populations of *P. lanceolata*. We analysed whether the phytopathogen infection affected performance of the butterfly larvae, the rate of successful parasitism by *C. melitaeorum*, the subsequent performance of the parasitoid offspring and the sex ratio of the parasitoid broods.

The experiment took place during the summer of 2005 and the spring of 2006. Nine gregarious families of second instar *M. cinxia* larvae (100–200 larvae) were each split in half and randomly assigned to be fed fungus-infected or uninfected *P. lanceolata* (figure 2). These families originated from a well-mixed laboratory colony of butterflies that were the progeny of individuals collected throughout Åland. The larvae were fed fresh leaf pieces daily. Because the early stage of fungal infection may be difficult to detect, yet already alter nutritional quality for *M. cinxia* (cf. Laine 2004b), we collected the healthy and fungus-infected leaves from different populations. This eliminated the possibility of accidentally feeding infected leaves to the larvae in the healthy diet treatment. In order to minimize confounding

plant population with diet treatment, we collected healthy leaves from multiple healthy populations, and infected leaves from multiple infected populations. Additionally, previous studies have shown that there is no population-level variation of *P. lanceolata* quality for *M. cinxia* (van Nouhuys *et al.* 2003). Furthermore, it is not at all probable that a *P. lanceolata* population is uninfected due to resistance to *P. plantaginis*. This is because in Åland there are multiple strains of the fungus, and while a plant may be resistant to particular strains, it is not resistant in an absolute sense (e.g. Laine 2004a, 2006).

When the larvae had reached fourth instar, three groups of 10 larvae from each family feeding on each diet were put into Petri dishes. The larvae were kept in groups because they are gregarious and cannot live independently during early instars. Two of the groups from each family in each treatment (fungus infected and uninfected) were randomly chosen to be parasitized by *C. melitaeorum*. One of these was for dissection before diapause, and the other was for assessing the survival, size, developmental rate and sex in the following spring. This resulted in 54 Petri dishes making up six treatments, each with nine replicate families: healthy diet; fungus-infected diet; healthy diet+parasitism; fungal diet+parasitism; healthy diet+parasitism for dissection; and fungal diet+parasitism for dissection (figure 2).

To parasitize the host larvae, 35 female *C. melitaeorum* were used. These wasps were the progeny of wasps collected from natural populations in the Åland Islands in the spring of 2005. Three days after emergence, each female was mated to a male from a different local population and then kept in an individual plastic vial and fed 1 : 3 honey water absorbed in cotton. Over a period of two weeks, the wasps were used in a rotation of several wasps per day, each parasitizing one to five larvae. With one exception, host larvae within a family were parasitized consecutively so that larvae from the fungal-infected and uninfected diet treatments were parasitized at nearly the same time. Each larva was observed being parasitized by a single wasp, and multiple wasps parasitized the larvae in each dish.

In order to compare the performance of host larvae from the different treatments, the larvae in each dish were counted and weighed at diapause (fifth instar), and the number of days it took to develop to diapause was recorded. When all of the larvae had reached diapause, one of the parasitized groups from each family in each diet treatment was chosen at random to be dissected in order to count the number of first instar *C. melitaeorum* larvae in each host larva. The remaining parasitized and unparasitized host larvae were left to diapause for the winter under sheltered outdoor conditions. The following spring the number of host larvae in each dish surviving through diapause was counted, and then the larvae were reared until the emergence of the *C. melitaeorum*. The number of *C. melitaeorum* pupating from each larva was counted, and each wasp pupa was weighed at 24 hours. The sex of adult wasps was recorded.

(c) Statistical analyses

(i) Effect of fungus-infected diet and parasitism on the larvae of *M. cinxia*

Data on the effect of fungus-infected diet and parasitism by *C. melitaeorum* on the *M. cinxia* larvae were analysed as generalized linear mixed models (GLMM). In all models, diet and parasitism were fixed factors, and larval family was included as a random variable. To test for interaction between

the treatments, the interaction term ‘fungus × parasitoid’ was included in all models. The response variables ‘development time’ and ‘weight’ were normally distributed. The overwintering survival of *M. cinxia* larvae (0/1) was analysed by fitting a bimodal distribution of errors.

(ii) Effect of host diet on the parasitoid *C. melitaeorum*

Data on the effect of host diet on *C. melitaeorum* were analysed as GLMMs with host family as a random variable. The effect of ‘family’ could not be estimated for *C. melitaeorum* brood size after diapause, the proportion of *C. melitaeorum* pupae that survived to adulthood or for the sex ratio of *C. melitaeorum* broods, because mortality during diapause resulted in an unbalanced data structure. These data were analysed as generalized linear models (GLM). The data on the number of *C. melitaeorum* larvae (brood size) found in the dissected larvae of *M. cinxia* in the autumn were normally distributed after clear outliers had been identified and omitted from the data (observations outside the range of $2 \pm \text{s.d.}$ around the group mean). Data on the size of *C. melitaeorum* broods emerging from host larvae in the spring (after larval diapause) had a Poisson distribution of errors and the proportion of the emerged *C. melitaeorum* pupae that survived to maturity was analysed assuming a binomial error distribution. The analysis of the weight of the *C. melitaeorum* pupae included the parasitoid sex and the interaction term ‘sex × fungus’ to test whether the weight of males and females responded differently to the fungal-infected diet treatment. The data on weights were normally distributed. Finally, the sex ratio of *C. melitaeorum* broods was analysed with a binomial distribution of errors.

(iii) *C. melitaeorum* metapopulation dynamics

If fungal-infected diet affects the performance of *C. melitaeorum* under laboratory conditions, then we might expect wasp performance at a landscape scale to be associated with the occurrence of *P. plantaginis* in natural populations of *P. lanceolata*. In order to test this, we analysed the population dynamics of *C. melitaeorum* in the Åland Islands with respect to fungal infection of *P. lanceolata*. In particular, we analysed whether the presence of the plant pathogen was associated with colonization, change in population size and extinction of local *C. melitaeorum* populations. Because previous studies have shown that habitat connectivity strongly influences the population dynamics of *C. melitaeorum* (Lei & Hanski 1997; van Nouhuys & Hanski 2002), all our statistical models included an estimate of connectivity, S_{ij} (Moilanen & Nieminen 2002), as an explanatory variable. S_{ij} takes into account the distances of each host population from host populations occupied by *C. melitaeorum*, and the sizes (number of *M. cinxia* larval groups) of those host populations.

We analysed *C. melitaeorum* colonization dynamics using survey records of transition of local butterfly populations from unoccupied to occupied by *C. melitaeorum*. In addition to connectivity (S_{ij}), the explanatory variables in the model were the presence of fungus in the population (as *C. melitaeorum* surveyed in the spring come from larvae parasitized in the previous autumn), year, host population size (the number of larval groups in the previous autumn) and latitude and longitude to account for possible spatially autocorrelated factors explaining colonization dynamics. The analysis of the effect of fungal infection on extinctions was based on *C. melitaeorum* populations that were occupied one year and then persisted to the next year or did not. The explanatory

Table 1. The effect of the fungus-infected host diet on the parasitoid *C. melitaearum*. (Wald's *Z*-statistic is given for random effects and the *F*-statistic is given for fixed effects.)

source	variance estimate for random effects	<i>Z/F</i>	<i>p</i>
<i>(a) no. of C. melitaearum in larvae dissected in autumn</i>			
family	0.11 ± 0.09	1.23	0.109
residual	1.08 ± 0.13	8.4	<0.0001
fungus _{1,141}		4.06	0.046
<i>(b) no. of C. melitaearum coming out of larvae in the spring</i>			
fungus _{1,20}		0.45	0.501
<i>(c) proportion of C. melitaearum surviving to maturity</i>			
fungus _{1,14}		0.65	0.434
<i>(d) weight of C. melitaearum</i>			
family	0.05 ± 0.04	1.37	0.086
residual	0.13 ± 0.03	4.85	<0.0001
sex _{1,47}		43.6	<0.0001
fungus _{1,47}		5.16	0.027
sex × fungus _{1,47}		0.26	0.616
<i>(e) sex ratio of C. melitaearum</i>			
fungus _{1,15}		4.81	0.045

variables were the same as in the model of colonizations except here we used host population size in the spring as *C. melitaearum* can only survive where their host also survived over the winter. Both extinctions and colonizations were analysed as GLMMs with binomially distributed errors. Population ID was included as a random variable.

The change in *C. melitaearum* population size (the difference in the number of parasitized nests between subsequent years) was analysed as a GLM with normally distributed errors. Explanatory variables in the model were presence of fungus, year, host population size (the number of larval groups in the spring) and latitude and longitude. In all models, non-significant interactions were excluded in a backward stepwise manner. All GLMMs were implemented with the GLMIX macro and all GLMs with the PROC GENMOD procedure in SAS v. 9.1 (SAS institute 1999). The models with a bimodal error distribution used a logit link function, and models with a Poisson distribution of errors a log link function.

3. RESULTS

(a) Effect of fungus-infected diet and parasitism on the larvae of *M. cinxia*

Feeding on fungus-infected leaves of *P. lanceolata* significantly slowed down the development of *M. cinxia* larvae prior to diapause (30.0 days ± 0.49 s.e. versus 31.4 days ± 0.68 s.e.; electronic supplementary material, table 1a). However, weight at diapause (5.7 mg ± 0.17 s.e. versus 5.6 mg ± 0.19 s.e.) was not significantly affected by the diet nor was it affected by parasitism by *C. melitaearum* (electronic supplementary material, table 1b). Fungus infection did not significantly alter overwintering survival of *M. cinxia* larvae, but fewer larvae parasitized by *C. melitaearum* survived over the winter than did unparasitized larvae. The negative effect of *C. melitaearum* on overwintering survival was marginally statistically significant (91% parasitized versus 96% unparasitized; $p=0.056$, electronic supplementary material, table 1c).

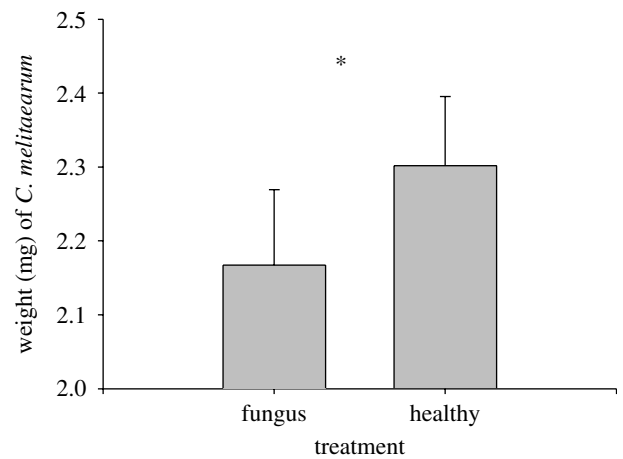


Figure 3. The weight of *C. melitaearum* parasitoids developing in *M. cinxia* larvae that had been fed mildew-infected host plants compared with those that had been fed healthy host plants. The error bars are based on standard errors of means. *Indicates a statistically significant difference at $p=0.027$.

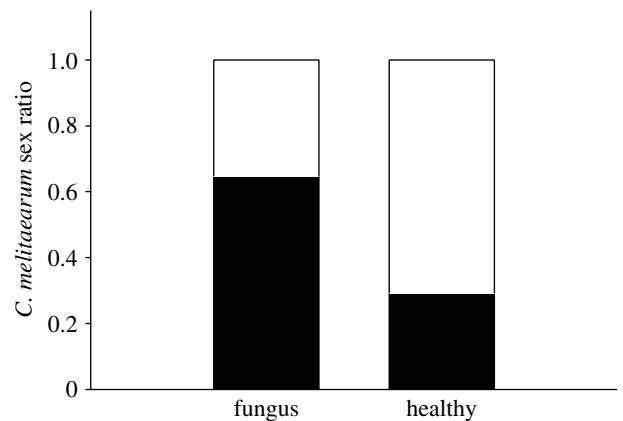


Figure 4. The mean sex ratios of *C. melitaearum* broods that emerged from *M. cinxia* larvae that had been fed mildew-infected host plants compared with those that had been fed healthy host plants (black bar, female).

(b) Effect of host diet on the parasitoid

Significantly larger broods of *C. melitaearum* were found in the larvae of *M. cinxia* dissected in the autumn that had been fed the fungus-infected diet compared with larvae fed healthy *P. lanceolata* (2.6 ± 0.12 s.e. versus 2.2 ± 0.13 s.e.; table 1a). After diapause, the brood sizes of the pupating *C. melitaearum* also tended to be higher in the hosts fed the fungus-infected diet, but the difference was not statistically significant (table 1b). Among the pupating *C. melitaearum*, host diet did not have a significant effect on survival to maturity (64% infected diet versus 54% healthy diet; table 1c). Parasitoids reared from hosts fed the infected diet weighed less than those from hosts fed a healthy diet ($2.30 \text{ mg} \pm 0.09$ s.e. versus $2.16 \text{ mg} \pm 0.10$ s.e.; table 1d; figure 3). Also, as is typical for Hymenoptera, the females were heavier than the males in both treatments ($2.57 \text{ mg} \pm 0.08$ s.e. versus $1.91 \text{ mg} \pm 0.07$ s.e.; table 1d). The sex ratio of the *C. melitaearum* broods was significantly altered by the host's diet, with highly female-biased broods emerging from larvae fed the fungal-infected diet (65% female versus 29% female; table 1e; figure 4). A female-biased sex ratio was observed in 75% of the broods emerging from larvae fed the infected diet,

Table 2. Factors affecting the colonization dynamics of *C. melitaeorum* analysed with generalized mixed linear models. (Wald's *Z*-statistic is given for random effects and the *F*-statistic is given for fixed effects.)

source	variance estimate for random effects	<i>Z</i> / <i>F</i>	<i>p</i>
population ID	4.64 ± 0.49	9.51	<0.0001
residual	0.17 ± 0.005	34.17	<0.0001
fungus _{1,1829}		32.94	<0.0001
host population size _{1,1829}		95.49	<0.0001
year _{1,1829}		8.95	0.003
latitude _{1,1829}		10.61	<0.001
longitude _{1,1829}		56.14	<0.0001
connectivity _{1,1829}		4.52	0.034
latitude × connectivity _{1,1829}		42.55	<0.0001
year × connectivity _{1,1829}		8.42	0.004

whereas none of the broods from larvae fed healthy leaves had a sex ratio above 50% female.

(c) Association of fungus infection with the population dynamics of the parasitoid

Colonization of local host populations by *C. melitaeorum* was more likely to occur in *P. lanceolata* patches infected by the powdery mildew than in uninfected patches (table 2). The probability of the wasp successfully colonizing a previously unoccupied *M. cinxia* population increased by more than twofold where the patch was occupied by *P. plantaginis*. That is, of the host butterfly populations without fungus, on average 3.2% were successfully colonized, whereas 7.1% host populations with fungus were successfully colonized. However, the presence of fungus had no significant effect on either the probability of extinction (electronic supplementary material, table 2a) or the change in *C. melitaeorum* population size (electronic supplementary material, table 2b). Not surprisingly, probability of colonization by *C. melitaeorum* (table 2) as well as change in *C. melitaeorum* population size (electronic supplementary material, table 2b) was positively associated with host population size. Colonization probability was also affected by the latitude and longitude of the population as well as the degree of connectivity (table 2). Extinction probability of local *C. melitaeorum* populations varied significantly only among years (electronic supplementary material, table 2a).

4. DISCUSSION

Many studies have shown that host quality influences parasitoid fitness under laboratory conditions (as reviewed by Ode 2006). Several studies have further demonstrated that rate of parasitism of a herbivore is affected by plant pathogens (Biere *et al.* 2002; Cardoza *et al.* 2003), and that host plant quality can affect parasitoid population dynamics (i.e. van Nouhuys & Hanski 1999). Our results are the first to indicate that the dynamics of natural parasitoid populations can be mediated by an indirect interaction with a plant pathogen. We demonstrate that the plant pathogen indirectly affects parasitoid adult weight and the sex ratio of parasitoid broods. Furthermore, a

compelling interpretation of our metapopulation data is that the plant pathogen facilitates the colonization of local host populations by the parasitoid. Given that *C. melitaeorum* occurs as a metapopulation with a high rate of turnover in a fragmented landscape, any factor affecting the colonization success of the parasitoid affects its regional persistence.

(a) Performance of the host and the parasitoid

In this study we found that under laboratory conditions feeding on fungal-infected diet decreased rate of development of *M. cinxia* larvae, but did not affect their ultimate size or rate of survival. This confirms a previous study in which *M. cinxia* larvae feeding on fungal-infected diet were also found to develop more slowly. However, in the previous study, larvae fed the fungal-infected diet weighed less than did larvae fed the healthy diet (Laine 2004b). Some of the discordance between the studies may be attributed to significant differences among *M. cinxia* families in their response to variation in diet quality (Laine 2004b). Slow development of a herbivore is an indication of low host quality, and may be detrimental under natural conditions as it prolongs the period of vulnerability to larval parasitoids and predators (Williams 1999), as well as (in this case) late summer drought, and early autumn freezing (Kuussaari *et al.* 2004). Diet was not associated with the overwintering survival of *M. cinxia* larvae. However, parasitism by *C. melitaeorum* increased overwintering mortality, as has been shown previously (van Nouhuys & Tay 2001; van Nouhuys & Hanski 2004).

Fungal infection of host diet apparently had both negative and positive effects on the parasitoid. Among the *M. cinxia* dissected before winter diapause, there were larger parasitoid broods in host individuals fed fungus-infected diet than uninfected diet. However, by the time the wasps matured to pupation in the following spring, there was no difference in the size of broods originating from larvae fed the two diets. The fact that initially the brood size in host larvae fed the infected diet was large suggests that the hosts were high quality, or that they were perceived by the ovipositing wasps as high quality. Alternatively, larger brood size may be the result of low immune response of the hosts fed the fungal-infected diet. That is, hosts fed the healthy diet may have successfully killed a fraction of *C. melitaeorum* eggs or small larvae.

Ultimately, the brood size was equal, and those developing in the larvae fed the fungal-infected diet were small, indicating that the larvae on the fungal-fed diet were poor quality hosts, and that the immature parasitoids developing in them experienced higher resource competition (Hardy *et al.* 1992). Though host larvae fed the two diets did not differ in size, stronger competition among brood-mates in the hosts fed the fungal-infected diet is possible. For instance, much of the carbon and nitrogen ingested by the host may be plant defensive compounds such as flavonoids and alkaloids that accumulate in the host but remain unavailable as food for the developing parasitoids. Because fitness of parasitoids is generally thought to increase with size, with large size associated with high longevity and fecundity (Godfray 1994), overall the fungus-infected diet may lower parasitoid fitness.

(b) Sex ratio of the parasitoid broods

Cotesia melitaearum, like all other Hymenoptera, is haplodiploid, with unfertilized eggs developing into males and fertilized eggs being female. Many factors influence whether a female wasp lays a fertilized or an unfertilized egg (summarized in Godfray (1994)). The sex ratio of a brood of *C. melitaearum* laid in a *M. cinxia* larvae naturally range from entirely male to entirely female (S. van Nouhuys 2003, unpublished data). Because the natural parasitoid populations are small (van Nouhuys & Tay 2001) and their dynamics are under long-term study (van Nouhuys & Hanski 2002), we do not know the natural spatial pattern of sex ratio variation in the system. In this study we found that the sex ratio of broods developing in larvae fed the infected diet tended to be female biased while broods developing in larvae fed the uninfected diet were male biased. Generally parasitoids are predicted (Charnov 1982), and have been observed, to have a female-biased sex ratio in higher quality hosts (i.e. Charnov *et al.* 1981; Fox *et al.* 1990; Ueno 1999). Further study is needed to understand the mechanism of the observed variation of sex ratio. As mentioned above, ovipositing *C. melitaearum* may tend to lay female eggs because they perceive larvae fed the infected diet as better hosts, even though we find that those larvae are not better hosts. Alternatively, there may be differential mortality favouring females, due to resource competition within the hosts. However, differential developmental mortality between parasitoid sexes has been explored in very few cases (reviewed in Hardy 1998), and where it has been measured, no difference has been found (i.e. Hardy & Cook 1995).

(c) Parasitoid metapopulation dynamics

All three species in this study, the herbivore, phytopathogen and parasitoid, have metapopulation dynamics with high rates of local turnover (van Nouhuys & Hanski 2002; Nieminen *et al.* 2004; Laine & Hanski 2006). This means that factors affecting rate of colonization are important for the persistence of the species, or at least for the stability and size of the metapopulation. For the parasitoid *C. melitaearum*, the effects of habitat connectivity, host plant species and host phenology have all been shown to affect rate of colonization, and subsequently the large scale population dynamics of the wasp (van Nouhuys & Hanski 1999, 2002; van Nouhuys & Lei 2004).

In this study we found that colonization of local host populations by *C. melitaearum* was positively associated with the presence of *P. plantaginis*. There are three possible interpretations of this association. First, colonization of the fungal-infected patches might tend to occur owing to some unmeasured factor that affects both species independently. If this is the case, then the landscape scale effect of the plant pathogen on the fitness and sex ratio of the wasp is mediated by yet another factor. A second interpretation is that colonization by *C. melitaearum* encourages persistence of the plant pathogen. This is unlikely because successful colonization by the plant pathogen is strongly affected by attributes of the plant as well as microclimatic variation and landscape configuration (Laine 2004a; Laine & Hanski 2006). The presence of *C. melitaearum* is unlikely to influence these variables. Finally, successful colonization by the parasitoid may be facilitated by the phytopathogen.

A plausible mechanism for the positive association of colonization by *C. melitaearum* and host plant fungal infection is through the effects of the fungus on parasitoid sex ratio. A female-biased sex ratio, as long as it is not too extreme, should result in a high intrinsic rate of increase. This would increase the probability of success of newly founded populations. It is probable that in Åland one or several individual female *C. melitaearum* immigrate to an unoccupied local host population and lay just a few eggs in a few larvae of the summer generation (Lei *et al.* 1997). The successful annual colonization (what we analysed) hinges on these progeny, which become adults in the late summer to parasitize more larvae in the same host population. A high proportion of females would increase the number of hosts parasitized, decreasing the large, primarily stochastic chance that the parasitoid fails to establish.

If the metapopulation dynamics of *C. melitaearum* is influenced by *P. plantaginis*, then factors that influence the metapopulation dynamics of the mildew should also affect the wasp at a landscape scale. For instance, climate change, making summers warmer and increasing rainfall (IPCC 2001), may increase the prevalence of *P. plantaginis* (cf. Ingram & Robertson 1999; Laine 2007), which would increase the metapopulation size of *C. melitaearum*. Alternatively, too little rain or too much heat might make *P. plantaginis* infection sparse in the landscape (cf. Ingram & Robertson 1999; Laine 2007), reducing the already marginal metapopulation size of *C. melitaearum* (van Nouhuys & Tay 2001; van Nouhuys & Lei 2004).

We have shown with an experimental study that a plant pathogen affects aspects of fitness and sex ratio of a specialist parasitoid wasp. Long-term survey of the spatial and temporal metapopulation dynamics of the parasitoid shows an association of colonization by *C. melitaearum* with the plant pathogen, which may be explained by the effect of the plant pathogen on the sex ratio of the parasitoid wasp. A multitrophic-level interaction such as this has not previously been observed, but may be common because it is well known that the pathogen infection affects plant quality, which affects herbivore quality, which in turn affects parasitoid fitness (including sex ratio). The metapopulation level effect of variation of parasitoid sex ratio should be explored more thoroughly, both theoretically and empirically.

We thank K. Fedrowitz, R. Kaartinen, C. Lindqvist and M. Ojanen for their laboratory assistance, Nåtö Biological Station and Ålands Naturbruksskola for laboratory facilities and A. Biere T. Roslin and three anonymous referees for their helpful comments on the manuscript. This work was funded by the Academy of Finland Centre of Excellence Programme grant nos. 20286 and 213457.

REFERENCES

- Agrios, G. N. 1997 *Plant pathology*. Berkeley, CA: Academic Press.
- Barbosa, P., Gross, P. & Kemper, J. 1991 Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology* **72**, 1567–1575. (doi:10.2307/1940956)
- Biere, A. & Honders, S. C. 2006 Coping with third parties in a nursery pollination mutualism: *Hadena bicurvis* avoids

- oviposition on pathogen-infected, less rewarding *Silene latifolia*. *New Phytol.* **169**, 719–727. (doi:10.1111/j.1469-8137.2005.01511.x)
- Biere, A., Elzinga, J. A., Honders, S. C. & Harvey, J. A. 2002 A plant pathogen reduces the enemy-free space of an insect herbivore on a shared host plant. *Proc. R. Soc. B* **269**, 2197–2204. (doi:10.1098/rspb.2002.2147)
- Biere, A., Marak, H. B. & van Damme, J. M. M. 2004 Plant chemical defence against herbivores and pathogens: generalized defence or trade-offs? *Oecologia* **104**, 430–441.
- Bos, M. 1992 Gene flow characters and population structure in *Plantago lanceolata*. In *Plantago: a multidisciplinary study* (eds P. J. C. Kuiper & M. Bos), pp. 222–231. Berlin, Germany: Springer.
- Bottrell, D. G., Barbosa, P. & Gould, F. 1998 Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annu. Rev. Entomol.* **43**, 347–367. (doi:10.1146/annurev.ento.43.1.347)
- Bultman, T. L., Mcniell, M. R. & Goldson, S. L. 2003 Isolate-dependent impact of fungal endophytes in a multitrophic interaction. *Oikos* **102**, 491–496. (doi:10.1034/j.1600-0706.2003.11477.x)
- Bushnell, W. R. 2002 The role of powdery mildew research in understanding host–parasite interaction: past, present and future. In *The powdery mildews—a comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik & L. W. Carver), pp. 1–12. St Paul, MN: The American Phytological Society.
- Cardoza, Y. J., Teal, P. E. A. & Tumlinson, J. H. 2003 Effect of peanut plant fungal infection on oviposition preference by *Spodoptera exigua* and on host-searching behavior by *Cotesia marginiventris*. *Environ. Entomol.* **32**, 970–976.
- Charnov, E. L. 1982 *The theory of sex allocation*. Princeton, NJ: Princeton University Press.
- Charnov, E. L., Los-Den Hartogh, R. L., Jones, W. T. & van Den Assem, J. 1981 Sex ratio evolution in a variable environment. *Nature* **289**, 27–33. (doi:10.1038/289027a0)
- Fox, L. R., Letourneau, D. K., Eisenbach, J. & van Nouhuys, S. 1990 Parasitism rate and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. *Oecologia* **83**, 414–419.
- Godfray, H. C. J. 1994 *Parasitoids: behavioural and evolutionary ecology*. Princeton, NJ: Princeton University press.
- Hardy, I. C. W. 1998 Patterns of sex ratio, virginity and developmental mortality in gregarious parasitoids. *Biol. J. Linn. Soc.* **64**, 239–270. (doi:10.1006/bijl.1998.0225)
- Hardy, I. C. W. & Cook, J. M. 1995 Brood sex ratio variance, developmental mortality and virginity in a gregarious parasitoid wasp. *Oecologia* **103**, 162–169. (doi:10.1007/BF00329076)
- Hardy, I. C. W., Griffiths, N. T. & Godfray, H. C. J. 1992 Clutch size in a parasitoid wasp: a manipulation experiment. *J. Anim. Ecol.* **61**, 121–129. (doi:10.2307/5515)
- Harvey, J. A., van Nouhuys, S. & Biere, A. 2005 Effects of quantitative variation in allelochemical in *Plantago lanceolata* on development of a generalist and a specialist herbivore and their endoparasitoids. *J. Chem. Ecol.* **31**, 287–302. (doi:10.1007/s10886-005-1341-1)
- Harvey, J. A., van Dam, N. M., Witjes, M. A., Soler, R. & Gols, R. 2007 Effects of dietary nicotine on the development of an insect herbivore, its parasitoid and secondary hyperparasitoid over four trophic levels. *Ecol. Entomol.* **32**, 15–23. (doi:10.1111/j.1365-2311.2006.00838.x)
- Hatcher, P. E. 1995 Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. *Biol. Rev.* **70**, 639–694.
- Ingram, D. & Robertson, N. 1999 *Plant disease—a natural history*. London, UK: Harper Collins Publishers.
- IPCC (Intergovernmental Panel Climate Change) 2001 *Climate Change 2001: impacts, adaptation, and vulnerability, contribution of working group II to the Intergovernmental Panel on Climate Change third assessment report*. Cambridge, UK: Cambridge University Press.
- Kankare, M. & Shaw, M. R. 2004 Molecular phylogeny of *Cotesia* (Hymenoptera: Braconidae: Microgastrinae) parasitoids associated with checkerspot butterflies. *Mol. Phylogenet. Evol.* **32**, 207–220. (doi:10.1016/j.ympev.2003.11.013)
- Kankare, M., van Nouhuys, S. & Hanski, I. 2005 Genetic divergence among host-specific cryptic species in *Cotesia melitaeorum* aggregate (Hymenoptera: Braconidae), parasitoids of checkerspot butterflies. *Ann. Entomol. Soc. Am.* **98**, 382–394. (doi:10.1603/0013-8746(2005)098[0382:GDAHCS]2.0.CO;2)
- Kuussaari, M., van Nouhuys, S., Hellmann, J. & Singer, M. C. 2004 Larval biology of checkerspots. In *On the wings of checkerspots: a model system for population biology* (eds P. R. Ehrlich & I. Hanski), pp. 138–160. Oxford, UK: Oxford University Press.
- Laine, A.-L. 2004a Resistance variation within and among host populations in a plant–pathogen metapopulation—implications for regional pathogen dynamics. *J. Ecol.* **92**, 990–1000. (doi:10.1111/j.0022-0477.2004.00925.x)
- Laine, A.-L. 2004b A powdery mildew infection on a shared host plant affects the dynamics of the Glanville fritillary butterfly populations. *Oikos* **107**, 329–337. (doi:10.1111/j.0030-1299.2004.12990.x)
- Laine, A.-L. 2006 Evolution of host resistance: looking for coevolutionary hotspots at small spatial scales. *Proc. R. Soc. B* **273**, 267–273. (doi:10.1098/rspb.2005.3303)
- Laine, A.-L. 2007 Pathogen fitness components and genotypes differ in their sensitivity to nutrient and temperature variation in a wild plant–pathogen association. *J. Evol. Biol.* **20**, 2371–2378. (doi:10.1111/j.1420-9101.2007.01406.x)
- Laine, A.-L. & Hanski, I. 2006 Large-scale spatial dynamics of a specialist plant pathogen in a fragmented landscape. *J. Ecol.* **94**, 217–226. (doi:10.1111/j.1365-2745.2005.01075.x)
- Lei, G. C. & Hanski, I. 1997 Metapopulation structure of *Cotesia melitaeorum*, a specialist parasitoid of the butterfly *Melitaea cinxia*. *Oikos* **78**, 91–100. (doi:10.2307/3545804)
- Lei, G. C., Vikberg, V., Nieminen, M. & Kuussaari, M. 1997 The parasitoid complex attacking the Finnish populations of Glanville fritillary *Melitaea cinxia* (Lep: Nymphalidae), an endangered butterfly. *J. Nat. Hist.* **31**, 635–648. (doi:10.1080/00222939700770301)
- Mattson, J. M. J. 1980 Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.* **11**, 119–161. (doi:10.1146/annurev.es.11.110180.001003)
- Moilanen, A. & Nieminen, M. 2002 Simple connectivity measures in spatial ecology. *Ecology* **83**, 1131–1145.
- Mondy, N. & Corio-Costet, M.-F. 2004 Feeding insects with a phytopathogenic fungus influences their diapause and population dynamics. *Ecol. Entomol.* **29**, 711–717. (doi:10.1111/j.0307-6946.2004.00642.x)
- Mook, J. H., Haeck, J., van Der Toorn, J. & van Tienderen, P. H. 1992 The demographic structure of populations. In *Plantago: a multidisciplinary study* (eds P. J. C. Kuiper & M. Bos), pp. 69–87. Heidelberg, Germany: Springer.
- Moran, P. J. & Schultz, J. C. 1998 Ecological and chemical associations among late-season squash pests. *Environ. Entomol.* **27**, 39–44.
- Nieminen, M., Siljander, M. & Hanski, I. 2004 Structure and dynamics of *Melitaea cinxia* metapopulations. In *On the wings of checkerspots: a model system for population biology* (eds P. R. Ehrlich & I. Hanski), pp. 63–91. Oxford, UK: Oxford University Press.

- Ode, P. J. 2006 Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol.* **51**, 163–185. (doi:10.1146/annurev.ento.51.110104.151110)
- Omacini, M., Chaneton, E. J., Ghersa, C. M. & Müller, C. B. 2001 Symbiotic fungal endophytes control insect host–parasite interaction webs. *Nature* **409**, 78–81. (doi:10.1038/35051070)
- Ovaskainen, O. & Laine, A.-L. 2006 Inferring evolutionary signals from ecological data in a plant–pathogen metapopulation. *Ecology* **87**, 880–891. (doi:10.1890/0012-9658(2006)87[880:IESFED]2.0.CO;2)
- Price, J. S., Bever, J. D. & Clay, K. 2004 Genotype, environment and genotype by environment interactions determine quantitative resistance to leaf rust (*Coleosporium asterum*) in *Euthamia graminifolia* (Asteraceae). *New Phytol.* **162**, 729–743. (doi:10.1111/j.1469-8137.2004.01082.x)
- Rodriguez-Saona, C., Chalmers, J., Raj, S. & Thaler, J. 2005 Induced plant responses to multiple damagers: differential effects on an herbivore and its parasitoid. *Oecologia* **143**, 566–577. (doi:10.1007/s00442-005-0006-7)
- Ross, M. D. 1973 Inheritance of self incompatibility in *Plantago lanceolata*. *Heredity* **30**, 169–176.
- SAS institute 1999 *SAS/STAT software user's guide*. Release 8.00. Cary, NC: SAS Institute, Inc.
- Slansky, F. & Wheeler, G. S. 1992 Caterpillars' compensatory feeding response to diluted nutrients leads to toxic allelochemical dose. *Entomol. Exp. Appl.* **65**, 171–186. (doi:10.1007/BF00221368)
- Stout, J. M., Thaler, J. S. & Thomma, B. P. H. J. 2006 Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annu. Rev. Entomol.* **51**, 663–689. (doi:10.1146/annurev.ento.51.110104.151117)
- Turlings, T. C. J. & Benrey, B. 1998 Effects of plant metabolites on the behaviour and development of parasitic wasps. *Ecoscience* **5**, 321–333.
- Ueno, T. 1999 Host-size-dependent sex ratio in a parasitoid wasp. *Res. Popul. Ecol.* **41**, 47–57. (doi:10.1007/PL00011982)
- Urrutia, C. M. A., Wade, M. R., Phillips, C. B. & Wratten, S. D. 2007 Influence of host diet on parasitoid fitness: unravelling the complexity of a temperate pastoral agroecosystem. *Entomol. Exp. Appl.* **123**, 63–71. (doi:10.1111/j.1570-7458.2007.00526.x)
- van Damme, J. M. M. 1992 Breeding systems in *Plantago*. In *Plantago: a multidisciplinary study* (eds P. J. C. Kuiper & M. Bos), pp. 12–18. Berlin, Germany: Springer.
- van Nouhuys, S. & Hanski, I. 1999 Host diet affects extinctions and colonizations in a parasitoid metapopulation. *J. Anim. Ecol.* **68**, 1248–1258. (doi:10.1046/j.1365-2656.1999.00365.x)
- van Nouhuys, S. & Hanski, I. 2002 Colonization rates and distances of a host butterfly and two specific parasitoids in a fragmented landscape. *J. Anim. Ecol.* **71**, 639–650. (doi:10.1046/j.1365-2656.2002.00627.x)
- van Nouhuys, S. & Hanski, I. 2004 Natural enemies of checkerspot butterflies. In *On the wings of checkerspots: a model system for population biology* (eds P. R. Ehrlich & I. Hanski), pp. 161–180. Oxford, UK: Oxford University Press.
- van Nouhuys, S. & Lei, G. C. 2004 Parasitoid and host metapopulation dynamics: the influences of temperature mediated phenological asynchrony. *J. Anim. Ecol.* **73**, 526–535. (doi:10.1111/j.0021-8790.2004.00827.x)
- van Nouhuys, S. & Tay, W. T. 2001 Causes and consequences of small population size for a specialist parasitoid wasp. *Oecologia* **128**, 126–133. (doi:10.1007/s004420100635)
- van Nouhuys, S., Singer, M. C. & Nieminen, M. 2003 Spatial and temporal patterns of caterpillar performance and the suitability of two host plant species. *Ecol. Entomol.* **28**, 193–202. (doi:10.1046/j.1365-2311.2003.00501.x)
- Vet, L. E. M. & Dicke, M. 1992 Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* **37**, 141–172. (doi:10.1146/annurev.en.37.010192.001041)
- Walters, D. R. & Ayres, P. G. 1981 Growth and branching pattern of roots of barley infected with powdery mildew. *Ann. Bot.* **47**, 159–162.
- Williams, I. S. 1999 Slow-growth, high-mortality: a general hypothesis, or is it? *Ecol. Entomol.* **24**, 490–495. (doi:10.1046/j.1365-2311.1999.00217.x)
- Yarwood, C. E. 1978 History and taxonomy of powdery mildews. In *The powdery mildews* (ed. M. D. Spencer), pp. 1–32. London, UK: Academic Press.