

Parasitism by the mite *Trombidium breei* on four U.K. butterfly species

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Abstract. 1. The incidence of parasitism by larvae of the mite species *Trombidium breei* was reported in one population of the lycaenid butterfly *Polyommatus icarus*, four populations of the satyrine butterfly *Maniola jurtina*, one population of the satyrine butterfly *Aphantopus hyperanthus*, and two populations of the satyrine butterfly *Pyronia tithonus*, as well as on one specimen of the dipteran *Alophorus hemiptera*. A considerable proportion of butterflies (11–50%) was infested in all study populations.

2. The pattern of infestation was examined in detail in *M. jurtina*. Males had a significantly higher incidence of infestation than females, and middle-aged butterflies had a higher incidence of infestation than old or young butterflies. The incidence of infestation peaked in the middle of the flight season, and this seasonal effect was independent of the effect of butterfly age.

3. Using a model based on capture–recapture data, it was estimated that a hypothetical ideal male *M. jurtina* that lives exactly the mean expected lifespan of 9–10 days has an approximately 75% chance of becoming infested with mites at least once during its lifetime, a mean time to first infestation of 3–4 days, and an average infestation persistence time of 2–3 days.

4. Capture–recapture data failed to show any effect of mite infestation on the lifespan or within-habitat movement rate of *M. jurtina*.

5. In experiments in which individual butterflies were taken out of their normal habitat and released, *M. jurtina* and *P. tithonus* that were infested with mite larvae did not differ from uninfested individuals in the efficiency with which they returned to suitable habitat. Thus, parasitism by *T. breei* larvae had no detectable effects on flight performance or orientation ability.

6. The results suggest that trombidiid mite larvae have limited potential in the biological control of insect pests.

Key words. *Atomus parasiticus*, butterfly lifespan, butterfly parasites, mite parasitism, pest control, trombidiid larvae.

Introduction

Adults of many European butterfly species, as well as of other insect species, are parasitised by bright red mite larvae of the family Trombidiidae (Acarina) (Southcott, 1986). Trombidiid larvae climb onto hosts when they alight on

plants (Zhang, 1992) and parasitise them by sucking the host's body fluids (exsanguination) and by using the host as a means of transport for dispersal (Pugh *et al.*, 1991; Zhang, 1998). It has been suggested that exsanguination and impairment of locomotion might be detrimental to the host's survival and reproduction (Pugh *et al.*, 1991), in which case trombidiid mites could be effective as a means of biological control of a variety of insect pests, including butterflies and moths (Welbourn, 1983; Zhang & Xin, 1989; Chen & Zhang, 1991; Pugh *et al.*, 1991; Weiberg & Edwards, 1997). The effect of parasitism by trombidiid

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mite larvae on individual insect hosts has not been studied under natural conditions, however, and little is known about the pattern of mite parasitism in specific host populations in the wild.

The work reported here arose from chance observations of parasitism by trombidid larvae on a population of U.K. butterflies that was being studied for other reasons (Conradt *et al.*, 2000). Contrary to expectation, the parasite in question was identified as *Trombidium (Teresothrombium) breei* (Trombididae: Prostigmata) (Southcott, 1986), rather than the supposedly more common *Trombidium (Trombidium) poriceps* (Evans *et al.*, 1961). The present investigation followed up this observation in four respects. (1) The generality of the previous observation was tested by recording the incidence of parasitism by trombidid larvae on meadow brown *Maniola jurtina* L. (Satyridae: Lepidoptera), ringlet *Aphantopus hyperanthus* L. (Satyridae: Lepidoptera), gatekeeper *Pyronia tithonus* L. (Satyridae: Lepidoptera), and common blue *Polyommatus icarus* L. (Lycaenidae: Lepidoptera) butterflies at four locations in the south of England. (2) Seven different aspects of the pattern of parasitism that had potential relevance to the issue of pest control were examined in detail, including the age and sex classes of adult butterflies that were parasitised, the time course of parasitism relative to the butterflies' flight period, and whether mite parasitism affected the movement of butterflies within suitable habitat. (3) A model was devised to estimate parasite attachment rates and durations on individual hosts, using field mark–release–recapture data. This model, which can be applied to other insect species for which mites are a potential means of biological control, allows estimation of the probability and duration of parasitism for individual hosts. (4) Flight experiments were conducted with individual butterflies in field conditions to determine whether flight and orientation were impaired by the presence of mites.

Methods

Study sites

The study was conducted in July and August 1999 and 2000 at four study sites, all of which consisted of unimproved, unmown calcareous grassland: (1) Newmarket: a 120 × 30 m section of the Devil's Dyke close to Newmarket, U.K. (52°12'N, 0°23'E); (2) Burwell Dyke: a section of the Devil's Dyke close to Burwell, U.K. (52°17'N, 0°19'E), of size 100 × 20 m in 1999 and 300 × 20 m in 2000; (3) Burwell Road: an 80 × 5 m roadside verge parallel to the Devil's Dyke close to Burwell (52°17'N, 0°19'E); and (4) Sussex: a 200 × 20 m downland site beside a golf course in Lewes, U.K. (50°52'N, 0°3'E).

Mark–release–recapture data

Butterflies were caught, sexed, and marked with a unique individual number code using a felt pen. According to their

wing-wear pattern, they were classified as young (hardly worn wings: nearly unimpaired wing fringes and nearly all scales present); middle-aged (medium worn wings: wing fringes impaired but most scales present); or old (very worn wings: strongly impaired wing fringes and many scales lost). The butterflies were also examined closely for mite larvae and classified as infested or uninfested. Because *T. breei* mite larvae are bright red and 1.3–2.1 mm long (Southcott, 1986), they were observed easily with the naked eye on the dark brown bodies of the butterflies. Butterflies were not infested with any other obvious ectoparasites. After examination, butterflies were either released at the capture point or used for flight experiments (see below). In 1999, 391 *M. jurtina* were captured, examined, and marked at Newmarket, 269 *M. jurtina* at Burwell Dyke, 33 *M. jurtina* at Burwell Road, and 44 *M. jurtina* and 10 *P. icarus* in Sussex. In 2000, 470 *M. jurtina*, 100 *A. hyperanthus*, and 97 *P. tithonus* at Burwell Dyke, and 116 *P. tithonus* at Burwell Road were examined and marked (not including recaptures).

Identification of mite larvae

The mite larvae of 24 infested *M. jurtina*, eight *A. hyperanthus*, 20 *P. tithonus*, and four *P. icarus*, chosen randomly from all of the study populations, were removed carefully, using a fine brush to nudge the mites gently until they dropped off the butterfly. Mite larvae were killed immediately and stored in 70% alcohol. They were mounted on slides in polyvinyl lactophenol and identified using Southcott (1986).

Flight experiments

Flight experiments on *M. jurtina* and *P. tithonus* used a procedure developed originally to investigate butterfly dispersal behaviour (see Conradt *et al.*, 2000). Butterflies were caught and released in unsuitable habitat (very short grass or harvested wheat field) at distances of 15–200 m from their capture point, which was in suitable habitat (long grass). Released butterflies flew off immediately in search of suitable habitat, whereupon they were followed on foot and the following characteristics of their flight behaviour were scored: estimated flight height on a scale of one to six (above plant level: ≤15 cm, 15–30 cm, 30–50 cm, 50 cm–1 m, 1–2 m, ≥2 m); estimated flight speed on a scale of one to three (slow, medium, fast); start speed (s per first 10 m); estimated flight path length (m); duration of flight (s); and flight type [either a relatively straight flight or a systematic search flight that involved returning in loops around the release site: see Conradt *et al.* (2000) for details and definitions]. Observations were terminated when butterflies had either returned successfully to suitable habitat or had moved >300 m from the release point. Flight orientation was measured in terms of angular difference between starting direction and habitat direction (initial orientation), angular difference between direction at termination of

observation and direction of habitat (final orientation), and the rate of successful returns to suitable habitat. Experimental data on each of these nine variables were collected for 206–299 *M. jurtina* butterflies, of which 54–78 butterflies were infested with *T. brevi* larvae; and for 80–137 *P. tithonus* butterflies, of which 14–23 were infested (exact sample sizes varied for different measures of flight behaviour, because it was not possible to record every aspect of behaviour in every individual). It was predicted that if mites were having a detrimental influence on their hosts, this would be revealed by impaired flight performance.

The data were analysed using separate multiple linear regression models for each aspect of the flight pattern and for each butterfly species. In each of these models, the following potentially confounding factors were first controlled for: release distance, butterfly sex, butterfly age, and environmental factors (wind speed and direction, sun direction, light intensity, cloud cover, the proportion of time for which the sun was behind clouds during a flight, temperature, and humidity). To control for the possibility that old butterflies were more affected by mite infestation, the analysis was repeated in *M. jurtina* on old butterflies only (sample sizes: 89–98 butterflies, of which 24–27 carried mites).

Within-habitat movement rates

During the course of a separate study (Conradt *et al.*, 2001), within-habitat movement distances were determined between marking and first recapture for 94 *M. jurtina* that had been caught and released within suitable habitat at Burwell Dyke over a period of 9 days. Movement distances were found to be independent of time elapsed until first recapture for recaptures ≥ 24 h after marking, allowing individuals to be counted as moved if they had moved ≥ 50 m, and as not moved if they had moved < 50 m from the site of first capture. The proportion of infested and uninfested butterflies that had moved between capture and recapture was used to determine the effect of mite infestation on within-habitat movement rates of *M. jurtina*.

Model to estimate butterfly survival/residence rate

The lifespan/residence time was estimated for *M. jurtina* butterflies at the Burwell Dyke study site using mark–release–recapture data. (Note that this method cannot distinguish between butterflies that died and butterflies that emigrated from the study area.) In 1999, 171 individual *M. jurtina* were marked and released over a period of 35 days at Burwell Dyke, of which 25 individuals were recaptured on 27 occasions. In 2000, 228 individual *M. jurtina* were marked over a period of 10 days at Burwell Dyke, of which 74 individuals were recaptured on 93 occasions. Because males and females did not differ in the time between recaptures, data were combined for both sexes. To predict the lifespan/residence time of butterflies, it was assumed that (1) population size was approximately constant during the

observation period (i.e. birth and immigration rates balanced death and emigration rates; because the observation periods were in the middle of the main flight period, when adult density is likely to have reached a plateau, this assumption seemed justified); (2) day-to-day survival/residence rate was approximately constant; and (3) marked and unmarked butterflies had the same probability of capture. Thus, the expected number of butterflies that was first caught and marked on day x and recaptured on day y [$R_x(y)$], was:

$$R_x(y) = \frac{m(y) + r(y)}{N} \cdot s^{y-x} \cdot m(x) \quad (1)$$

where $m(y)$ = number of new captures on day y , $r(y)$ = number of recaptures on day y , $m(y) + r(y)$ = total number of captures on day y , N = population size, $[m(y) + r(y)]/N$ = probability for a butterfly to be captured on day y , s = day-to-day probability of survival/residence at study site, s^{y-x} = probability of survival/continued residence from day x to day y , $m(x)$ = number of new captures on day x , $s^{y-x}[m(x)]$ = number of butterflies that were first caught on day x and survived until day y .

The expected total number of recaptures $R(y)$ on day y was therefore:

$$R(y) = \sum_{x=1}^{y-1} R_x(y) = \frac{m(y) + r(y)}{N} \cdot s^y \cdot \sum_{x=1}^{y-1} \frac{m(x)}{s^x} \quad (2)$$

whereby $m(y)$, $m(x)$, and $r(y)$ can be determined directly from the mark–release–recapture data. $f(s, N)$ was chosen as a measure of discrepancy between the expected [$R(y)$] and the observed [$r(y)$] number of recaptures on each day. s and N were estimated using a computer program that changed the values for s and N stepwise until it reached a minimum for $f(s, N)$:

$$f(s, N) = \sum_{y=1}^{35} [R(y) - r(y)]^2 \quad (3)$$

Using the estimated value for s , the mean expected lifespan/residence time of butterflies was calculated as:

$$E(\text{lifespan}) = \sum_{t=1}^{\infty} s^t (1-s) \cdot t = \frac{s}{1-s} \quad (4)$$

where t = age in days, $s^t(1-s)$ = probability to survive to and die at age t .

Model to estimate rate of mite acquisition and persistence

Recapture data from male *M. jurtina* were used to estimate the average time taken for an uninfested butterfly to become infested with mite larvae and the average time for

which mites resided on butterflies before they dropped off. In 1999, 31 recapture events could be used, with recapture spans of 1–16 days. Twenty-four butterflies had no mites at first capture, of which six acquired mites before recapture, and seven butterflies had mites at first capture, of which three had lost their mites at recapture. In 2000, 86 recapture events could be used, with recapture spans of 1–9 days. Seventy-five butterflies had no mites at first capture, of which 16 acquired mites before recapture, and 11 butterflies had mites at first capture, of which four had lost their mites at recapture. Because mite larvae are often aggregated on vegetation (Pugh *et al.*, 1991) and butterflies will often pick up more than one larva on a single occasion, the number of mites per butterfly was not considered; butterflies were classified as either infested or uninfested. It was also assumed that an uninfested butterfly had a probability w of acquiring mite larvae from one day to the next, and an infested butterfly had a chance l of losing its mite larvae from one day to the next. Thus, the probability that an initially uninfested butterfly had become infested at recapture after z days, was:

$$\begin{aligned} P_z(\text{none} \rightarrow \text{mites}) &= P_{z-1}(\text{none} \rightarrow \text{none}) \cdot w \\ &+ P_{z-1}(\text{none} \rightarrow \text{mites}) \cdot (1-l) \\ &= \frac{w}{w+l} \cdot [1 - (1-w-l)^z] \end{aligned} \quad (5)$$

where $P_z(\text{none} \rightarrow \text{none})$ = probability that an originally uninfested butterfly has no mites at recapture after z days, $P_z(\text{none} \rightarrow \text{mites})$ = probability that an originally uninfested butterfly has acquired mites on recapture after z days, $P_z(\text{none} \rightarrow \text{none}) = 1 - P_z(\text{none} \rightarrow \text{mites})$.

Similarly, the probability was calculated that an infested butterfly had become uninfested at recapture after z days, as:

$$\begin{aligned} P_z(\text{mites} \rightarrow \text{none}) &= P_{z-1}(\text{mites} \rightarrow \text{none}) \cdot (1-w) \\ &+ P_{z-1}(\text{mites} \rightarrow \text{mites}) \cdot l \\ &= \frac{l}{w+l} \cdot [1 - (1-w-l)^z] \end{aligned} \quad (6)$$

where $P_z(\text{mites} \rightarrow \text{mites})$ = probability that an originally infested butterfly still has mites at recapture after z days, $P_z(\text{mites} \rightarrow \text{none})$ = probability that an originally infested butterfly has lost its mites on recapture after z days, $P_z(\text{mites} \rightarrow \text{mites}) = 1 - P_z(\text{mites} \rightarrow \text{none})$.

Equations 5 and 6 were used to predict, for each observed recapture period, the likelihood that an originally uninfested butterfly would become infested, and that an originally infested butterfly would lose its mites, as functions of w and l . These likelihoods were used to calculate the predicted total number of butterflies that should have changed from uninfested to infested $T(\text{none} \rightarrow \text{mites})$ and from infested to uninfested $T(\text{mites} \rightarrow \text{none})$ in the sample of recaptured butterflies (according to observed recapture periods), again as functions of w and l . w and l were then estimated by a computer program that varied the values of w and l

until a best fit was reached between the predicted numbers of butterflies that changed mite status and the observed numbers [$O(\text{none} \rightarrow \text{mites})$ and $O(\text{mites} \rightarrow \text{none})$]. This occurred when $g(w, l)$ became minimal:

$$\begin{aligned} g(w, l) &= [T(\text{none} \rightarrow \text{mites}) - O(\text{none} \rightarrow \text{mites})]^2 \\ &+ [T(\text{mites} \rightarrow \text{none}) - O(\text{mites} \rightarrow \text{none})]^2 \end{aligned} \quad (7)$$

Analogous to eqn 4, the day-to-day survival rate of butterflies (s), the day-to-day probability of acquiring mite larvae (w), and the day-to-day probability of losing mite larvae (l) were used to calculate the mean expected time taken for an uninfested butterfly to acquire mite larvae before dying:

$$\begin{aligned} E(\text{acquiring rate}) &= \sum_{t=1}^{\infty} (1-w)^{t-1} \cdot w \cdot s^t \cdot t \\ &= \frac{w \cdot s}{[1 - (1-w) \cdot s]^2} \end{aligned} \quad (8)$$

and the mean expected time that a mite resides on a butterfly (until either the mite drops off or the butterfly dies):

$$\begin{aligned} E(\text{persistence duration}) &= \sum_{t=1}^{\infty} [(1-l)^t \cdot l \cdot s^t \\ &+ (1-l)^t \cdot s^t \cdot (1-s)] \cdot t \\ &= \frac{(1-s+l) \cdot [(1-l) \cdot s]}{[1 - (1-l) \cdot s]^2} \end{aligned} \quad (9)$$

The expected proportion of butterflies that never became infested during their lifetime was estimated as:

$$\begin{aligned} E(\text{proportion of butterflies never infested}) &= \sum_{t=1}^{\infty} (1-w)^t \cdot s^t \cdot (1-s) \\ &= \frac{(1-s)(1-w)s}{1 - (1-w)s} \end{aligned} \quad (10)$$

Results

Identification of mite larvae

The commonest trombidid mite larva to be found on butterflies in the U.K. is generally considered to be *Trombidium (Trombidium) poriceps* (Oudemans, 1904) syn. *Atomus parasiticus* de Geer (Evans *et al.*, 1961); however Southcott (1986) described the larva of another species, *Trombidium (Teresothrombium) breei*, which is very similar morphologically to *T. poriceps* and was found on marbled

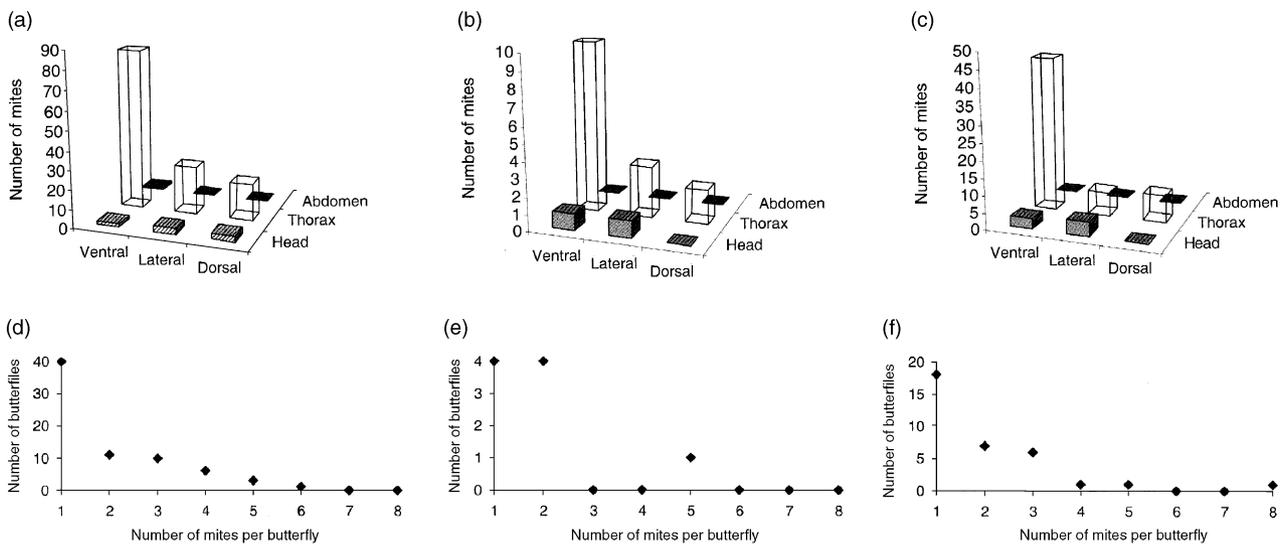


Fig. 1. Frequency distribution of *Trombidium brei* larvae that were attached to various body parts of butterflies in the three satyrine butterfly species: (a) *Maniola jurtina*, (b) *Aphantopus hyperanthus*, (c) *Pyronia tithonus*; and frequency distribution of number of mites per butterfly: (d) *M. jurtina*, (e) *A. hyperanthus*, (f) *P. tithonus*.

white butterflies *Melanargia galathea* L. (Satyridae: Lepidoptera) in Eastbourne (U.K.). Southcott (1986) distinguished larvae of *Trombidium* (*Tesothrombium*) *breei* from those of *Trombidium poriceps* on the basis of the thinning of the anterior part of the scutum, its lack of pores, and the presence there of poorly marked striations visible in some specimens. All of the mites that were collected from *M. jurtina*, *P. icarus*, *P. tithonus*, and *A. hyperanthus* showed the thin, poreless anterior region of the scutum described by Southcott (1986) and striations were visible in some individuals. It was concluded that the mite larvae in this study all belonged to the species *Trombidium brei*.

Affected body parts of butterflies

Trombidium brei larvae were almost always attached to the thorax of butterflies, and they were more often attached ventrally than laterally or dorsally (Fig. 1a–c). One to eight larvae were observed per infested butterfly (mean number of mites per infested butterfly \pm SE: *M. jurtina* 1.9 ± 0.2 , $n = 71$; *A. hyperanthus* 3.4 ± 0.4 , $n = 9$; *P. tithonus* 2.0 ± 0.3 , $n = 34$; Fig. 1d–f).

Overall rate of infestation

Overall infestation rates are given in Table 1. After potentially confounding factors (butterfly sex and age, location, year and date: see below) had been controlled for, there was no significant difference in infestation rate among the three satyrine butterfly species (logistic regression model, *t*-tests, pairwise comparisons, new significance level: $\alpha = 0.017$, d.f. = 1517: *M. jurtina*/*A. hyperanthus*: $t = 0.81$, $P > 0.1$, NS; *M. jurtina*/*P. tithonus*: $t = -0.48$, $P > 0.1$, NS; *A. hyperanthus*/*P. tithonus*: $t = -0.95$, $P > 0.1$, NS). Sample sizes for *P. icarus* were too small to enable similar comparisons to be made.

Detailed pattern of parasitism in *Maniola jurtina*

Because there was no significant difference between populations of *M. jurtina* once other effects had been controlled for ($\chi^2 = 0.6$, d.f. = 3, $n = 1207$, $P > 0.5$, NS), data were combined for all four populations. The infestation rate of *M. jurtina* varied significantly between years, seasonally, and between sexes and age classes (Table 2, Fig. 2a–f). On average, 2.5 times more males and 2.8 times more females

Table 1. Overall mite infestation rates in four butterfly species.

Butterfly species	Proportion of butterflies that was infested with mites (%)	Total number of butterflies examined
<i>M. jurtina</i>	23.7	1207
<i>A. hyperanthus</i>	11.0	100
<i>P. tithonus</i>	27.8	213
<i>P. icarus</i>	50.0	10

Table 2. Results of logistic multiple linear regression models of mite incidence on sex and age of butterflies, year, date, and population, and interactions of these factors, for *Maniola jurtina* (only significant results are shown; $n = 1207$).

Independent variables in the model	Test statistic	Parameter value (\pm SE) (logistic)	Mean residual deviance and degrees of freedom
+ sex	$\chi^2 = 19.2$, d.f. = 1, $P < 0.0001$	Males: -0.64 ± 0.19 Females: -1.86 ± 0.21	1.096, 1206 1.080, 1205
+ age	$\chi^2 = 6.8$, d.f. = 2, $P < 0.05$	Young: 0.00 ± 0.19 Middle aged: $+0.63 \pm 0.18$ Old: $+0.36 \pm 0.22$ Pairwise comparisons (new significance level: $\alpha = 0.017$, d.f. = 1203): young–middle: $t = -3.58$, $P < 0.001$; young–old: $t = -1.63$, $P = 0.10$, NS; middle–old: $t = 1.51$, $P = 0.13$, NS	1.068, 1203
+ year	$\chi^2 = 54.9$, d.f. = 1, $P < 0.001$	1999: 0.00 ± 0.22 2000: -1.22 ± 0.17	1.047, 1202
+ date	$\chi^2 = 38.0$, d.f. = 1, $P < 0.001$	-0.006 ± 0.001	1.013, 1201

were infested in 1999 than in 2000. In both years, the proportion of infested butterflies peaked around 21 July, i.e. in the middle of the flight season of adult *M. jurtina* (Asher, 1994). Male butterflies had a significantly higher frequency of infestation than females throughout the flight period (on average 1.9 times as high; Table 2). Middle-aged butterflies of both sexes had the highest incidence of parasitism, and this was significantly higher (by 1.6 times, on average; see Table 2) than the incidence of mite larvae on young butterflies. The incidence of mite larvae on old butterflies was intermediate between that of young and middle-aged animals. The high incidence of mite larvae on middle-aged butterflies was not a by-product of the seasonal trend in mite incidence: although the proportion of middle-aged butterflies peaked at around the same time as the overall incidence of mite larvae (Table 3), the effect of butterfly age on parasitism rate was independent of date ($\chi^2 = 0.3$, d.f. = 2, $n = 1207$, $P > 0.5$, NS). The incidence of mite larvae peaked over time independently within each age class (Fig. 2a–f).

Parasite acquisition and persistence rates

Only for *M. jurtina* at Burwell Dyke were sample sizes large enough to make the following estimates. In 1999, the mean recapture span for *M. jurtina* at Burwell Dyke was 7.2 days (range: 1–16 days, median: 7 days, $n = 25$). A daily population size of $N = 285$ was estimated and a day-to-day survival/residence probability for individual butterflies of $s = 0.90$. Thus, the estimated median of lifespan/residence time of butterflies was 6.6 days (IQR: 2.7–13.2 days). In 2000, when the study area was larger than in 1999, a population size of $N = 500$ was estimated, a day-to-day survival/residence probability of $s = 0.91$, and a median of life span/residence time of 7.3 days (IQR: 3.1–14.7 days). Because the

estimates of lifespan/residence time were similar to former estimates of lifespan for *M. jurtina* (≈ 7 days: C. D. Thomas, pers. comm.), and did not differ significantly between years despite the much larger area sampled in 2000, it is assumed in the following that they represent true lifespan estimates rather than residence times.

In 1999, infested and uninfested butterflies had similar recapture spans (infested butterflies: mean = 10.2 days, range = 5–16 days, median = 10 days, $n = 5$; uninfested butterflies: mean = 6.5 days, range = 1–16 days, median = 6 days, $n = 20$). They also had similar recapture rates in both years (1999: infested butterflies: 11.1%, $n = 45$; uninfested butterflies: 18.1%, $n = 116$; test for difference between infested and uninfested butterflies: $\chi^2 = 1.2$, d.f. = 1, $P > 0.2$, NS; 2000: infested butterflies: 30.6%, $n = 36$; uninfested butterflies: 34.6%, $n = 217$; test for difference: $\chi^2 = 0.2$, d.f. = 1, $P > 0.2$, NS; data for both years combined: $\chi^2 = 2.6$, d.f. = 1, $n_1 = 81$, $n_2 = 333$, $n_{\text{total}} = 414$, $P > 0.1$, NS; Fig. 3a). Thus, there was no indication that infested butterflies had shorter lifespans than uninfested butterflies in either year.

For uninfested male *M. jurtina* at Burwell Dyke, the estimated probability of acquiring mite larvae within a day (w) was 0.136 in 1999 and 0.144 in 2000, and the estimated probability of losing mite larvae (l) for infested males was 0.186 in 1999 and 0.278 in 2000. Thus, it was estimated that the proportion of butterflies that never became infested with mite larvae during its lifetime was 35.0% in 1999 and 31.7% in 2000. In addition, butterflies were predicted to be infested with mite larvae on average for a total of only 2.2 days (1999) and 1.8 days (2000) during their lifetime. Relative to a butterfly's expected lifespan of ≈ 7 days, this means that a butterfly carries mite larvae on average for less than a third of its adult life. Butterflies that became infested at least once acquired their mites on average after 2.5 ± 2.7 (SD) days in 1999 and after 2.7 ± 3.0 (SD) days in 2000; and

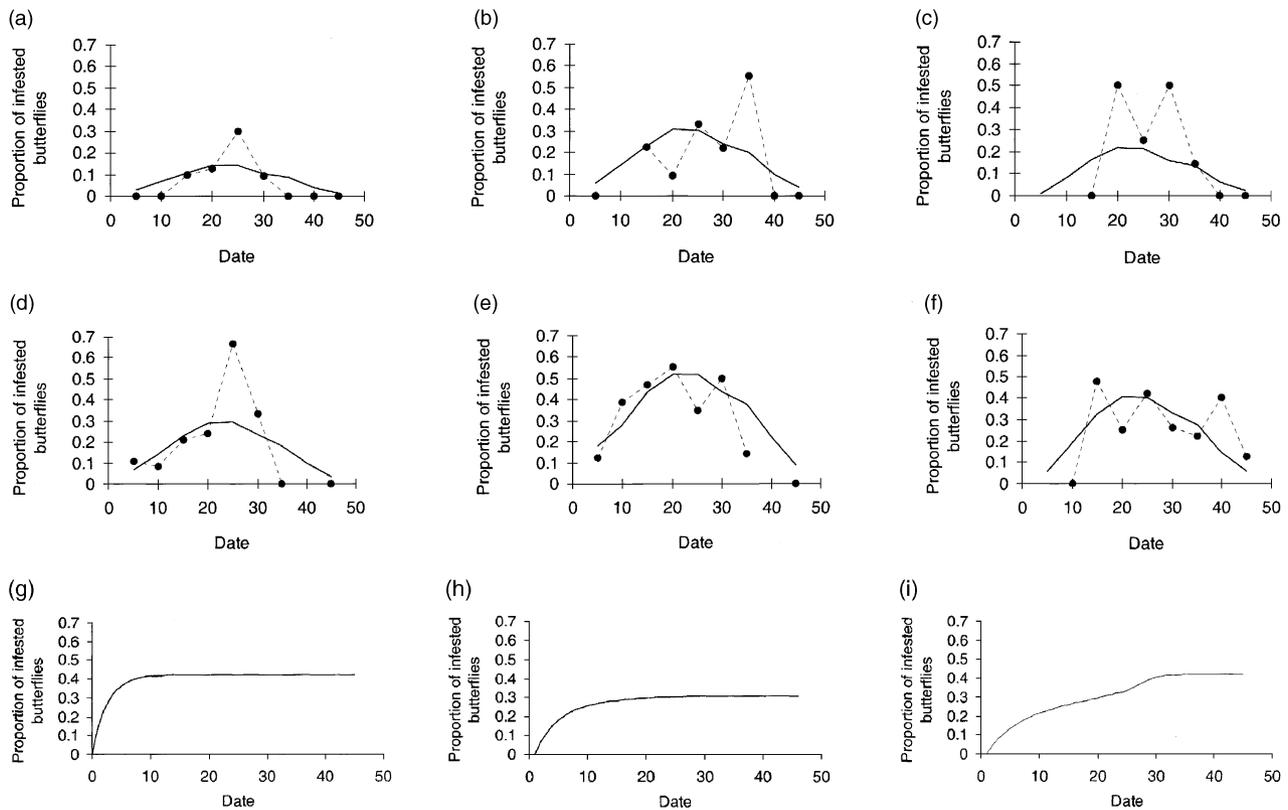


Fig. 2. Seasonal pattern of the proportion of *Maniola jurtina* of different age and sex that was infested with mites over the study period in 1999 (date 1–43 refers to 1 July to 12 August 1999): (a) young females, $n = 111$, (b) middle-aged females, $n = 76$, (c) old females, $n = 29$, (d) young males, $n = 131$, (e) middle-aged males, $n = 249$, (f) old males, $n = 141$. The filled circles are the observed proportions during 5-day intervals; the solid line represents the fitted regression line from the logistic model. Note that data for 2000 and for the other butterfly species are not shown because sample sizes were too small and/or sampling periods too short to investigate reliably the seasonal pattern of mite infestation rate. Predicted infestation rate versus date for 1999 is given in (g) within an age cohort of male *M. jurtina*, (h) within the male population, assuming a constant emergence rate of adult butterflies, and (i) within the male population, assuming an emergence rate of adult male butterflies that increases, peaks, then decreases over the flight period.

the mite larvae persisted on them (until the mites dropped off or the butterfly died) on average for 2.9 ± 3.1 (SD) days in 1999 and for 2.1 ± 2.2 days in 2000. For a hypothetical *ideal* male butterfly that has exactly the median expected lifespan of 7 days, the chance of becoming infested with mites at least once during its lifetime was 64% (1999) or 66% (2000), its expected mean time to first mite infestation was 3.4 days (both years), and the estimated average persistence time of mite infestation was 1.8 (1999) or 1.7 (2000) days.

Predicted time pattern of infestation rate

The model of acquisition and loss of mite larvae by *M. jurtina*, which was parameterised by male *M. jurtina* recapture data, predicted a time pattern of infestation rate, within each male age cohort, that started low early in the butterflies' lifetime and increased to a constant equilibrium proportion of 42% (1999) and 34% (2000) of butterflies being infested at any one time (see Fig. 2g). In the modelled

Table 3. Mean observation dates (\pm SE) for *Maniola jurtina* of different age and sex that were sampled between 1 July and 12 August 1999. The dates differed significantly among age classes (linear regression model, $F_{2,734} = 73.5$, $P < 0.001$) and the two sexes ($F_{1,733} = 105.5$, $P < 0.001$), but there was no interaction between age and sex ($F_{2,731} = 0.2$, $P > 0.5$, NS): young butterflies of both sexes were observed significantly earlier than middle-aged butterflies ($t = 4.1$, $P < 0.001$), and middle-aged butterflies were observed significantly earlier than old butterflies ($t = 6.6$, $P < 0.001$). Further, females were observed significantly later than males ($t = 7.1$, $P < 0.001$).

	Males	Females
Young	12.7 (± 0.7) days, $n = 131$	20.7 (± 0.9) days, $n = 111$
Middle aged	16.7 (± 0.5) days, $n = 249$	23.7 (± 1.2) days, $n = 76$
Old	26.7 (± 0.7) days, $n = 141$	2.8 (± 1.6) days, $n = 29$

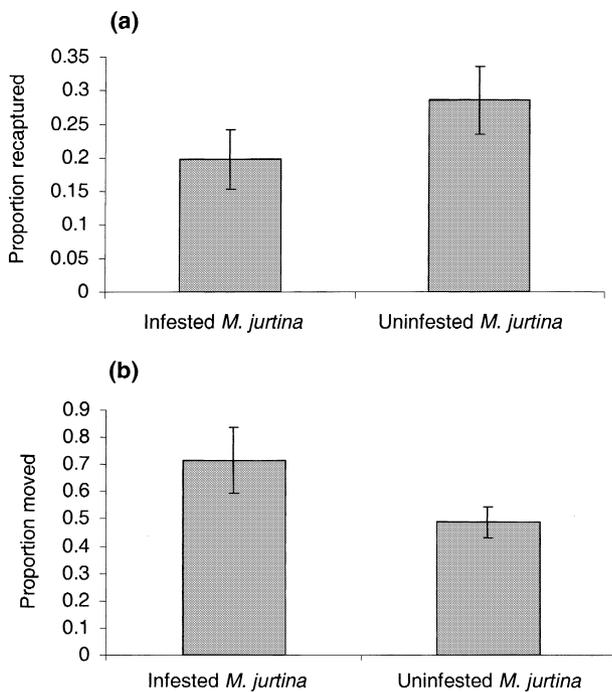


Fig. 3. (a) Recapture rates and (b) within-habitat movement rates of *Maniola jurtina*, depending on the mite infestation status of the butterflies; the error bars denote binomial standard deviations.

male population as a whole, the predicted infestation rate increased over time in a similar but slower pattern and either peaked or increased depending on whether the emergence rate of butterfly adults was constant (Fig. 2h), then decreased over the flight period (Fig. 2i). The model did not predict the drop of mite infestation rate that was observed later in the season (compare Figs 2d–f and g–i).

Influence of parasitism on butterflies' flight performance and movement rates

Presence or absence of mite infestation had no influence on the orientation or flight pattern of individual butterflies in either *M. jurtina* or *P. tithonus*: infested and uninfested individuals did not differ with respect to any of the parameters measured (3×9 multiple linear regression models: all F -values ≤ 3.0 , all $P > 0.1$, all NS). This result held for all age and sex classes of butterflies and after controlling for environmental conditions (see methods). Mite-infested *M. jurtina* had slightly, but not significantly, higher within-habitat movement rates than uninfested individuals ($\chi^2 = 2.45$, d.f. = 1, $n = 14$, $m = 80$, $P > 0.05$, NS; Fig. 3b).

Discussion

Trombidium brei larvae were only recently described in detail as parasites on the satyrine butterfly *M. galathea* (Southcott, 1986) and were not reported in the U.K.

before then. The results showed considerable levels of infestation with *T. brei* larvae in adults of three further satyrine butterfly species (*M. jurtina*, *A. hyperanthus*, *P. tithonus*) and one lycaenid species (*P. icarus*) at four study sites in the south of England. *Trombidium brei* infestation was also recorded serendipitously in a single specimen of the dipteran *Alophorus hemiptera*. It is generally supposed that the commonest trombidid mite larva to be found on butterflies is *Trombidium poriceps* (Evans *et al.*, 1961) but Southcott (1986) stated that *T. brei* larvae are very similar morphologically to *T. poriceps* larvae and at first misidentified his sample of *T. brei* larvae as *T. poriceps*. It is therefore likely that, in the past, *T. brei* larvae have sometimes been recorded as *T. poriceps*, and that parasitism by *T. brei* larvae is more widespread than is generally believed.

In *M. jurtina*, the only one of the butterfly species for which sufficient data were available, a significant seasonal pattern of infestation rate was observed, whereby the proportion of infested *M. jurtina* peaked in the third week of July. This is similar to the seasonal abundance pattern of adult *M. jurtina*, as well as that of *M. galathea* (Asher, 1994). It cannot be concluded with certainty, however, that the abundance of *T. brei* larvae in the environment peaked at the same time as the infestation rate on *M. jurtina*. For example, the data indicate that mite larvae may prefer middle-aged over old *M. jurtina*, and, therefore, mite infestation rate (but not environmental abundance of mite larvae) may peak at the time of peak abundance of middle-aged butterflies. If this were the case, infestation rate should differ between host species and could peak for each host species at the host species' own peak in abundance (e.g. see the relatively high infestation rate in the middle of August of *P. icarus*, the second flight season of which peaks at the end of August, 1 month later than that of *M. jurtina*). Such a synchrony in peak host abundance and infestation rate would favour the potential of mite larvae as biological control agents in a broad range of host species (Welbourn, 1983; Pugh *et al.*, 1991).

It is estimated that a significant proportion of an adult male *M. jurtina*'s reproductive lifespan in the wild is spent free of infestation. The estimates of mite attachment duration were close to those observed in laboratory studies of larvae of other mite species parasitising dipteran hosts (on average 3 days; Edwards & Dimmock, 1995). If these results are representative of other trombidid mite larvae and other parasitised insect species, the brief attachment duration would reduce the effectiveness of trombidid larvae as potential biological control agents considerably. In addition, infestation rates were lower in 2000, when weather conditions were unfavourable for adult butterfly activity, than in 1999, when weather conditions were favourable (Conradt *et al.*, 2000). This suggests that parasitism is less effective at a time when the host species has to withstand unfavourable environmental conditions, so that these two effects would not act synergistically to control host numbers. It was also found that female *M. jurtina* had lower infestation rates than males, which would reduce the effectiveness of mites as control agents further. Higher

parasitism rates in males than in females are believed to be a general pattern in vertebrate species (Zuk & McKean, 1996; Wedekind & Jacobsen, 1998) though it has recently been questioned whether the same is true of invertebrates (Sheridan *et al.*, 2000).

Surprisingly, the presence of mite larvae on *M. jurtina* and *P. tithonus* did not affect the butterflies' orientation or flight performance, or *M. jurtina*'s lifespan or within-habitat movement rate. Thus, the mites did not have any detectable effect on their hosts. The main attachment site of mite larvae on the ventral side of the thorax (approximately at the centre of gravity of the host's body) might in part explain this result, because this attachment site would result in little impairment of wing movement or gravitational balance. Laboratory studies on larvae of the mite *Unionicola foili* (Unionicolidae: Acari) also indicated no effect of parasitism on longevity or reproductive output of the hosts *Chironomus tentans* (Chironomidae: Diptera) (Weiberg & Edwards, 1997). Pugh *et al.* (1991) reported serious locomotor impairment and detrimental effects of exsanguination by *T. poriceps* larvae on *Chersodromia speculifera* Walker (Empididae: Diptera) but this study involved only a single host individual.

The work reported here produced the first detailed data on parasitism patterns of *Trombidium brevi* larvae under unmanipulated field conditions, including details of their effects on their hosts' performance; the implications of these patterns for the potential of mite larvae as biological control agents have been discussed. Further, the presented model is useful in estimating mite acquisition and persistence rates directly from unmanipulated field data. Because of limitations of the data, the model was used to estimate average rates but does not include age- or date-specific differences in rates; however, the close correspondence of the results between the 2 years, and relative to estimates collected by other studies under laboratory conditions, suggests that the estimates are fairly reliable, even without age- and date-specific details. Future authors with the requisite data could easily extend the model to include more details.

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References

Asher, J. (1994) *Butterflies of Berkshire, Buckinghamshire and Oxfordshire*. Pisces Publications, Oxford.

- Chen, P.R. & Zhang, Z.Q. (1991) Biology of *Allothrombium pulvinum* Ewing (Acari, Trombidiidae) and its impact on two-spotted spider mite (Acari, Tetranychidae) in cotton fields. *Journal of Applied Entomology*, **112**, 31–37.
- Conrath, L., Bodsworth, E.J., Roper, T.J. & Thomas, C.D. (2000) Non-random dispersal in the meadow brown butterfly (*Maniola jurtina*): implications for metapopulation models. *Proceedings of the Royal Society, B*, **267**, 1505–1510.
- Conrath, L., Roper, T.J. & Thomas, C.D. (2001) Dispersal behaviour of individuals in metapopulations of two British butterflies. *Oikos*, **95**, 416–424.
- Edwards, D.D. & Dimmock, R.V. (1995) Life history characteristics of larval *Unionicola foili* (Acari, Unionicolidae) parasitic on *Chironomus tentans* (Diptera, Chironomidae). *Journal of Natural History*, **29**, 1197–1208.
- Evans, G.O., Sheals, J.G. & MacFarlane, D. (1961) *The Terrestrial Acari of the British Isles*. British Museum, London.
- Pugh, P.J.A., King, P.E. & Fordy, M.R. (1991) Larvae of *Trombidium (Trombidium) poriceps* (Oudemans) (Trombidiidae: Prostigmata: Acarina) parasitic on *Chersodromia speculifera* Walker (Empididae: Diptera: Hexapoda). *Acarologia*, **22**, 139–155.
- Sheridan, L.A.D., Poulin, R., Ward, D.F. & Zuk, M. (2000) Sex differences in infections among arthropod hosts: is there a male bias? *Oikos*, **88**, 327–334.
- Southcott, R.V. (1986) Studies on the taxonomy and biology of the subfamily Trombidiinae (Acarina: Trombidiidae) with a critical revision of the genera. *Australian Journal of Zoology, Supplementary Series*, **123**, 1–116.
- Wedekind, C. & Jacobsen, P.J. (1998) Male-biased susceptibility to helminth infection: an experimental test with a copepod. *Oikos*, **81**, 458–462.
- Weiberg, M. & Edwards, D.D. (1997) Survival and reproductive output of *Chironomus tentans* (Diptera, Chironomidae) in response to parasitism by larval *Unionicola foili* (Acari, Unionicolidae). *Journal of Parasitology*, **83**, 173–175.
- Welbourn, W.C. (1983) Potential use of trombidid and erythraeid mites as biological control agents of insects. *Biological Control of Pests by Mites* (ed. by M. A. Hoy, G. L. Cunningham and L. Knutson), pp. 103–140. University of California Special Publications no. 3304, University of California Press, Oakland, U.S.A.
- Zhang, Z.Q. (1992) Phototactic and geotactic responses in *Allothrombium pulvinum* larvae (Acari, Trombidiidae). *Experimental and Applied Acarology*, **15**, 41–47.
- Zhang, Z.Q. (1998) Biology and ecology of trombidid mites (Acari, Trombidiidae). *Experimental and Applied Acarology*, **22**, 139–155.
- Zhang, Z.Q. & Xin, J.L. (1989) Biology of *Allothrombium pulvinum* (Acariformes: Trombidiidae), a potential biological control agent of aphids in China. *Experimental and Applied Acarology*, **6**, 101–108.
- Zuk, M. & McKean, K.A. (1996) Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology*, **26**, 1009–1023.

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