

This article was downloaded by: [informa internal users]

On: 16 March 2009

Access details: Access Details: [subscription number 755239602]

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Natural History

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title-content=t713192031>

The parasitoid complex attacking coexisting Spanish populations of *Euphydryas aurinia* and *Euphydryas desfontainii* (Lepidoptera: Nymphalidae, Melitaeini)

Constantí Stefanescu ^a; Josep Planas; Mark R. Shaw ^b

^a Butterfly Monitoring Scheme, Museu de Granollers-Ciències Naturals, Granollers, Spain ^b National Museums of Scotland, Edinburgh, UK

Online Publication Date: 01 March 2009

To cite this Article Stefanescu, Constantí, Planas, Josep and Shaw, Mark R.(2009)'The parasitoid complex attacking coexisting Spanish populations of *Euphydryas aurinia* and *Euphydryas desfontainii* (Lepidoptera: Nymphalidae, Melitaeini)', *Journal of Natural History*, 43:9,553 — 568

To link to this Article: DOI: 10.1080/00222930802610444

URL: <http://dx.doi.org/10.1080/00222930802610444>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

The parasitoid complex attacking coexisting Spanish populations of *Euphydryas aurinia* and *Euphydryas desfontainii* (Lepidoptera: Nymphalidae, Melitaeini)

Constantí Stefanescu^{a*}, Josep Planas^b and Mark R. Shaw^c

^aButterfly Monitoring Scheme, Museu de Granollers-Ciències Naturals, Granollers, Spain; ^bc/Pont, 26, E-08650 Sallent, Spain; ^cNational Museums of Scotland, Edinburgh, UK

(Received 30 November 2007; final version received 9 November 2008)

We report on a five-year investigation of the complex of parasitoids associated with coexisting populations of *Euphydryas aurinia* and *Euphydryas desfontainii* at a Spanish site. A sample of over 7000 eggs, 1000 larvae and 200 pupae (the last of these in part having been experimentally placed) revealed the existence of no egg parasitoids, of three primary larval parasitoids and of five primary pupal parasitoids, plus 13 secondary and facultatively tertiary parasitoids associated with the cocoons of the main larval parasitoid, the specialist *Cotesia* sp. D. The most abundant secondary parasitoid, *Neochrysocharis albiscapus*, entirely escaped tertiary parasitism by being gregarious. The mortality induced by parasitoids on the butterfly populations, although heavy, was clearly partially minimized by secondary parasitoids acting on *Cotesia* sp. D. The striking coincidence of the parasitoid complexes attacking the two *Euphydryas* species suggests a good system for investigating apparent competition between hosts sharing common natural enemies.

Keywords: butterflies; *Euphydryas aurinia*; *Euphydryas desfontainii*; parasitoid complex; secondary parasitism

Introduction

Almost all terrestrial insect communities include parasitoids, which potentially have important influences both in the population dynamics of their hosts and in the structure of the community [e.g. several papers in Waage and Greathead (1986) and in Hawkins and Sheehan (1994)]. The relationships between hosts and parasitoids within communities are frequently linked in complex food webs with further potentially structuring influences arising from the presence of hyperparasitoids (Müller et al. 1999; Morris et al. 2001, 2004; Lewis et al. 2002).

Much knowledge has been gained on the ecology of Melitaeini butterflies as a result of intensive research carried out in the last four decades (Ehrlich and Hanski 2004). From the earliest investigations, it became obvious that parasitoids were an important part of their complex of natural enemies (Stamp 1984; Moore 1989; Lei et al. 1997) and it was suggested that they could play a predominant role in the population dynamics of some Melitaeini species (Ford and Ford 1930).

Most of this work has focused on the extreme specialist larval parasitoids in the genus *Cotesia* (Hymenoptera: Braconidae), of which gregarious species are known from almost every Melitaeini species that has been studied in any detail (Eliasson

*Corresponding author. Email: canliro@teleline.es

and Shaw 2003; van Nouhuys and Hanski 2004). Recent molecular studies have revealed that throughout Europe, and no doubt Eurasia, there are many cryptic species of *Cotesia* associated with Melitaeini, each parasitoid having a very narrow host range (Kankare and Shaw 2004). Indeed, when a similar study was intensively focused on a small area of northeast Spain harbouring a particularly rich assemblage of Melitaeini, it was shown that with one exception each *Cotesia* species occurred in association with a single butterfly species (Kankare et al. 2005a). The only clear exception to this pattern was provided by the closely related *Euphydryas aurinia* and *Euphydryas desfontainii*, which are locally unique in sharing the same species of *Cotesia*.

Apart from *Cotesia*, little is known about the species of parasitoids comprising the parasitoid complexes of Melitaeini butterflies. From the few systems that have been studied (notably the Finnish populations of *Melitaea cinxia*; van Nouhuys and Hanski 2004) it can be deduced that, apart from *Cotesia* wasps, these butterflies are attacked by a substantial range of egg, larval and pupal primary parasitoids (Stamp 1981, 1984; White 1986; Komonen 1997, 1998; Wahlberg et al. 2001). Moreover, research on *M. cinxia* in Finland has demonstrated the existence of complex tritrophic interactions (i.e. secondary parasitoids attacking primary parasitoids; van Nouhuys and Hanski 2000, 2005; van Nouhuys and Tay 2001).

In this paper we present new data on the parasitoid assemblages found in association with coexisting populations of *E. aurinia* and *E. desfontainii* at a Spanish site within a larger area inhabited by several other Melitaeini, a system substantially different from that of Finnish *M. cinxia*. An effort was made to cover not only larval parasitoids but also egg and pupal parasitoids, and secondary parasitoids associated with *Cotesia* wasps. The knowledge attained over 5 years of field research should provide a good context for future investigations of the community structure and population dynamics of Melitaeini butterflies and their primary and secondary parasitoids. Moreover, it may prove useful for studying patterns of multitrophic interactions using comparative data from parasitoid complexes of *E. aurinia* populations from different geographical areas (e.g. potentially species-poor temperate areas in northern Europe versus potentially species-rich Mediterranean areas).

Material and methods

Study site and butterfly species

This study was carried out in the area known as El Guix, near Sallent, in Catalonia, northeastern Spain. This area is situated 500 m above sea level and is characterized by a Mediterranean climate, with mean maximum temperature in July of 30.0°C and mean minimum temperature in January of -0.8°C. Yearly average rainfall is 668 mm, mostly concentrated in autumn and spring; there is usually a severe period of summer drought between June and August, which leads to a hydric deficit of 100–200 mm.

Large populations of *E. aurinia* and *E. desfontainii* occur in abandoned vineyards on calcareous soil that have been colonized by vast expanses of the grass *Brachypodium retusum* and shrub vegetation. The host plants of *E. aurinia*, the honeysuckles *Lonicera implexa* and *Lonicera etrusca* (Caprifoliaceae), and of *E. desfontainii*, *Cephalaria leucantha* (Dipsacaceae), are also extremely abundant and

grow in close spatial association. Occasionally, and after depletion of the oviposition host plant, postdiapause larvae of *E. desfontainii* can be found feeding on *L. implexa*.

The two butterfly species have very similar life cycles in the study area. They are univoltine and adults can be found on the wing from early May to late June. Females lay egg clusters on the undersides of leaves of the host plants and the eggs hatch after about 3 weeks. In our study area, egg cluster size was 217.1 ± 100.8 eggs (mean \pm SD) in *E. aurinia* ($n=30$) and 123.5 ± 61.5 eggs in *E. desfontainii* ($n=8$). In *E. aurinia* different females may lay their eggs on the same plants and even on the same leaves, leading to merged gregarious larval groups that are much larger than the size of egg clusters laid by one female (Singer et al. 2002; Eliasson and Shaw 2003; Stefanescu et al. 2006). This phenomenon seems to occur only exceptionally in *E. desfontainii* (J. Planas, personal observation).

Larvae live in communal webs during the first three instars in July–August. After moulting to their fourth instar they enter diapause inside a denser silken web, usually hidden in the litter beneath the host plant. The larvae resume feeding in February–March, their gregarious behaviour becoming weaker by then and disappearing completely by the sixth and last instar. Pupation takes place in the litter or under rocks and stones, the pupal stage usually lasting about 3 weeks.

Sampling of parasitoids

Host parasitoids were sampled between 2001 and 2007 but the most intensive field work was carried out between 2002 and 2004. We combined the searching and sampling of immature stages in the field with an experimental approach to study pupal parasitism, as detailed below.

Egg parasitoids

In 2007, we assessed egg parasitism from 32 naturally laid egg clusters (26 from *E. aurinia* and six from *E. desfontainii*) located in the field. This sample was complemented with another six egg clusters (four from *E. aurinia* and two from *E. desfontainii*) obtained from captive females and transferred to the field after 1 to 5 days. Egg clusters were tagged, revisited periodically and brought to the laboratory the week after turning from cream to purple in colour, i.e. a few days before hatching. They were then kept inside transparent vials until hatching. To estimate cluster size, as well as losses from predation and possible parasitism, egg clusters were photographed with a digital camera when first discovered and when brought into the laboratory. Egg parasitism was assessed both from pictures showing eggs turning black (Stamp 1981) and from the rearing of possible parasitoids inside the transparent vials.

Larval parasitoids

Preliminary investigations of larval parasitism of *E. aurinia* and *E. desfontainii* were carried out in El Guix in 2001–2002. This first study showed that both *Euphydryas* species were regularly parasitized by the same species of *Cotesia*, which was identified as *Cotesia melitaeorum* agg. sp. D (Kankare et al. 2005a). Throughout the rest of this paper, this cryptic species is simply referred to as *Cotesia* sp. D.

In 2001–2002 and in 2004 we collected multiple samples, each of about 10 to 20 larvae, of *E. aurinia* and *E. desfontainii* from different nests and reared them in the laboratory to assess larval parasitism and to obtain pupae for the experiments on pupal parasitism (see below). In 2001–2002 we sampled both prediapause (4 July to 5 August 2001) and postdiapause larvae (24 February to 26 May 2002), but in 2004 we only sampled postdiapause larvae (7 March to 9 May). In total, we collected 619 larvae of *E. aurinia* (199 prediapause and 420 postdiapause larvae, from 46 nests) and 780 larvae of *E. desfontainii* (399 prediapause and 381 postdiapause larvae, from 56 nests). However, during the rearing process a significant fraction died for unknown reasons, believed to be unrelated to parasitism, so the effective sample size was considerably reduced: 369 larvae of *E. aurinia* (123 prediapause and 246 postdiapause larvae), and 436 larvae of *E. desfontainii* (256 prediapause and 180 postdiapause larvae).

Prediapause and early postdiapause larvae were collected from inside the web structure of the nest. Late postdiapause larvae were collected haphazardly from loose aggregations feeding or resting on host plants. To avoid a bias in the estimate of the incidence of parasitism (as the result of altered host behaviour rendering parasitized larvae more conspicuous; e.g. Shapiro 1976), we collected very few late sixth-instar single larvae (only two for each species).

The number of *Cotesia* broods emerging from host larvae, as well as the sizes of broods, were recorded from the above collections. In addition, in 2002–2004 several single postdiapause larvae bearing cocoons of *Cotesia* sp. D were collected from the field. Field collection could lead to an underestimation in the number of cocoons per larva if some cocoons had already been dislodged or taken by predators when first located in the field. To minimize this problem, two outliers (two sixth-instar larvae of *E. aurinia* showing abnormally low numbers of cocoons of one and seven, respectively) were excluded from the analysis.

Hyperparasitism of Cotesia sp. D.

We also recorded secondary parasitoids attacking *Cotesia* wasps. No true hyperparasitoids (those attacking the primary parasitoid while it is still growing inside the host) appeared in our samples. However, pseudohyperparasitoids (those attacking the primary parasitoid after it has finished feeding) were frequently reared from *Cotesia* cocoons, which is the stage normally attacked by pseudohyperparasitoids. Data on secondary parasitoids were obtained from the collection, in 2002–2004, of 424 cocoons of *Cotesia* sp. D belonging to 62 different masses found in association with the host remains (17 larvae in 2002, 36 larvae in 2003, and nine larvae in 2004). We have not tried to distinguish between secondary parasitoids associated with *E. aurinia* or *E. desfontainii* because, at least for the second postdiapause generation of the wasp (i.e. the one suffering the most severe hyperparasitism; see below), *Cotesia* larvae erupt and make their cocoons in places that are indistinguishable and not directly linked with either *Lonicera* spp. or *Cephalaria leucantha*. On the other hand, we have compared secondary parasitism of the two temporally distinct postdiapause emergences of *Cotesia* sp. D because they differ greatly in cocoon cluster size.

Data from 2003 and 2004 were also used to estimate the percentage of *Cotesia* that managed to emerge from cocoon masses in which parasitism of some of the

cocoons was recorded. Nothing emerged from a small fraction of cocoons. In these cases it was assumed that the unemerged *Cotesia* or pseudohyperparasitoids inside had died as a result of external factors (e.g. failure in our rearing process), and the cocoons were opened and the species present were scored as if they had emerged. The fate of cocoons from which an insect had already emerged at the time of collection was readily determined because *Cotesia* species cut a neat apical cap from their cocoon to emerge, whereas all the hyperparasitoids chew more irregular holes.

Pupal parasitoids

Between 2002 and 2004, 478 pupae resulting from wild-collected *E. aurinia* and *E. desfontainii* larvae in El Guix were experimentally placed in the field to investigate pupal mortality. They were placed, either as prepupae (40%) or as 1-day-old pupae (60%), in locations where wild pupae had been recorded previously. The fate of pupae was checked every other day and, after a period of about 15 days, those that had not disappeared as a result of predation were brought back to the laboratory to record parasitism (causes of mortality other than parasitism will be reported elsewhere; C. Stefanescu et al., in preparation). Levels of pupal parasitism were estimated considering only this subsample, after excluding pupae producing the tachinid *Erycia furibunda* (which, in fact, is a larval-pupal parasitoid; see below).

Additional data on pupal parasitoids were gathered from 22 wild pupae collected between 2002 and 2005 and six prepupae experimentally placed in El Guix in 2006.

Results

Egg parasitoids

Not a single case of egg parasitism was recorded in our sample of 6513 eggs of *E. aurinia* and 988 eggs of *E. desfontainii*. On the other hand, it was estimated that four out of 36 egg clusters in *E. aurinia* and one out of eight egg clusters in *E. desfontainii*, lost eggs through predation. In those cases, missing eggs represented $13.2 \pm 13.0\%$ of the egg cluster (amounting to only 1.4% of the overall egg sample).

Larval parasitoids

Three species of larval parasitoids were recorded from *E. aurinia* and *E. desfontainii* (Table 1; see also Kankare et al. 2005a). *Cotesia* sp. D was reared from both species, while the tachinids *Erycia furibunda* and *Compsilura concinnata* were reared in small numbers only from *E. aurinia*.

In the study area, *Cotesia* sp. D is a multivoltine species with recorded emergences from prediapause and postdiapause larvae of both hosts. This, together with the strong differences in the number of cocoons produced by the early and late collected postdiapause larvae, suggests that there are (at least partially) three generations (Table 2). Consequently, in August, before host larvae enter diapause, a few cocoons were found in larval nests (mean number of cocoons per parasitized larvae taking both hosts together, 2.3 ± 1.3 ; $n=4$ larvae). Cocoons were found again soon after host larvae had abandoned their diapause and resumed their feeding activity, mainly in March or early April. The number of parasitoids per parasitized larva increased slightly to an average of 3.2 ± 2.3 ($n=29$ larvae, taking both hosts together). This generation corresponds to those parasitoids that have overwintered

Table 1. Number of larvae of *Euphydryas aurinia* and *Euphydryas desfontainii* that were collected to assess parasitism, and incidence of the three recorded larval parasitoids in the host populations.

| | No. of groups | No. of larvae | Larvae/group Mean \pm SD | survival | Causes of mortality | | | | Parasitism by <i>Cotesia</i> sp. D | | |
|--------------------------------|---------------|---------------|-------------------------------|----------|-------------------------|-------------------------------------|---------------------------------------|---------|------------------------------------|-------------------------|-------------------------------------|
| | | | | | <i>Cotesia</i> sp. D | <i>Erycia</i> <i>furibunda</i> * | <i>Compsilura</i> <i>concinata</i> | Unknown | % larval mortality | % groups parasitized | % parasitism within groups |
| <i>Euphydryas aurinia</i> | | | | | | | | | | | |
| 2001 (prediapause) | 9 | 199 | 22.1 \pm 3.2 | 117 | 3 | 2 | 1 | 76 | 2.4 | 22.2 | 8.6 |
| 2002 (postdiapause) | 14 | 190 | 13.6 \pm 7.4 | 109 | 6 | 1 | 1 | 73 | 5.1 | 21.4 | 52.1 |
| 2004 (postdiapause) | 23 | 230 | 10.0 \pm 4.1 | 123 | 6 | 0 | 0 | 101 | 4.7 | 21.7 | 57.0 |
| <i>Euphydryas desfontainii</i> | | | | | | | | | | | |
| 2001 (prediapause) | 21 | 399 | 19.0 \pm 2.8 | 253 | 3 | 0 | 0 | 143 | 1.2 | 9.5 | 9.2 |
| 2002 (postdiapause) | 19 | 236 | 12.4 \pm 5.8 | 113 | 6 | 0 | 0 | 117 | 5.0 | 26.3 | 62.0 |
| 2004 (postdiapause) | 16 | 145 | 9.1 \pm 4.1 | 59 | 2 | 0 | 0 | 84 | 3.3 | 6.3 | 50.0 |

*Impact of *Erycia furibunda*, a larva–pupal parasitoid which emerges from the host pupa, was estimated from the pupal experiments (see Table 5).

Table 2. Number of *Cotesia* sp. D emerging from larvae of *Euphydryas aurinia* and *Euphydryas desfontainii*.

| | No. cocoons per host | | | |
|---|---------------------------|----------|--------------------------------|----------|
| | <i>Euphydryas aurinia</i> | | <i>Euphydryas desfontainii</i> | |
| | Mean \pm SD | <i>n</i> | Mean \pm SD | <i>n</i> |
| Prediapause larvae | | | | |
| Summer (July–August) | 1.0 \pm 0 | 1 | 2.7 \pm 1.2 | 3 |
| Postdiapause larvae | | | | |
| Late winter–early spring (January to mid-April) | 3.2 \pm 2.3 | 26 | 2.7 \pm 2.1 | 3 |
| Late spring (mid-April to early June) | 28.3 \pm 10.2 | 9 | 22.8 \pm 6.7 | 25 |

inside the host larvae and emerged from late fourth-instar or early fifth-instar larvae. Finally, a third generation was completed by late April and during May. Parasitoids erupted from old sixth-instar larvae, the average number of cocoons per host caterpillar increasing dramatically to an average of 24.2 ± 8.0 ($n=34$ larvae). The mean number of parasitoids from *E. aurinia* (28.3 ± 10.2) was slightly higher than from *E. desfontainii* (22.8 ± 6.7), although the difference was only marginally significant (t -test: 1.85, $p=0.07$, $n=9$ for *E. aurinia*, $n=25$ for *E. desfontainii*).

The whole phenological pattern may be more complex if, as recorded for *C. melitaeorum* agg. from Finnish *Melitaea cinxia* (S. van Nouhuys, personal communication), part of the first wave of *Cotesia* parasitizing prediapause caterpillars stay in their host through diapause until the following early spring. In that case, cocoons collected in early spring would consist of a mixture of first and second generations. Porter (1983) also noted that the prediapause eruption of *Cotesia bignellii* parasitizing a British population of *E. aurinia* was only partial.

The percentage of larval groups that suffered attacks by *Cotesia* sp. D remained stable at around 20% in *E. aurinia*, but varied between 6% and 26% in *E. desfontainii* (Table 1). However, the differences in the level of observed parasitism between the two species were not significant (χ^2 -test, $p>0.1$ in all three sampling periods). Interestingly, in both species there was a clear pattern of much lower levels of parasitism within parasitized larval groups in prediapause than in postdiapause larvae (10% versus 55%, approximately), although this may be explained by the (probably) only partial eruption of prediapause larvae.

The incidence of *Cotesia* sp. D from both host species was similar, and ranged from 1.2 to 2.4% of prediapause larvae and from 3.3 to 5.1% of postdiapause larvae (Table 1). As found by Porter (1983) for *C. bignellii* parasitizing *E. aurinia* in England and Lei et al. (1997) for a *C. melitaeorum* agg. species parasitizing *M. cinxia* in Finland, there seemed to be an increase in the incidence of parasitism between the two postdiapause emergences of the parasitoid, with percentages of parasitism in sixth-instar larvae more than double those recorded in fourth-instar or fifth-instar larvae (2001: 5.5% versus 1.7%; 2004: 5.7% versus 2.1%, respectively). However, our sample size was too small for statistical testing and we did not attempt to separate the two generations in Table 1.

Larvae of *E. aurinia* were also attacked by a second specialist parasitoid, the tachinid *Erycia furibunda*. Although *Erycia furibunda* attacks young host larvae before diapause, it emerges as an adult from the pupa or it leaves the pupa just before making its puparium (it is a larva–pupal parasitoid). As all host pupae resulting from our rearings were used in exposure experiments, its rate of parasitism was estimated from the pupal experiments reported in the next section (see also Table 5). In 2002 it showed an incidence of 11.1% in the hosts sampled, but in 2003 this figure decreased to 4.5%. In 2004 it was not recorded from the experimental pupae, although it appeared in two out of nine wild *Euphydryas* sp. pupae. Although not recorded from unquestionable *E. desfontainii* in our study, it is also a known parasitoid of that host (Ford et al. 2000)

Finally, the broad generalist tachinid *Compsilura concinnata* was reared once from an *E. aurinia* prepupa collected as a postdiapause larva. Before this rearing, *Compsilura concinnata*, a very common and widespread species in Europe, had apparently not been recorded from *E. aurinia* (H.-P. Tschorsnig, personal communication), which suggests that this represents an uncommon association and that *Compsilura concinnata* probably achieves a very low rate of parasitism in this host.

Hyperparasitism of *Cotesia* sp. D

Of the 62 cocoon masses found in the field in association with host remains, 35 produced secondary parasitoids (or tertiary parasitoids, see below) instead of primary *Cotesia* sp. D parasitoids.

For the 3-year period, we recorded an incidence of pseudohyperparasitism in cocoon masses of 56.5% (Table 3). However, both in 2002 and 2003 the incidence increased between the two postdiapause appearances of *Cotesia*, from 37.5–42.9% of the masses in early spring to 66.7–77.3% in late spring. The percentage of pseudohyperparasitism within parasitized cocoon masses was high and surprisingly stable, ranging from 83.4 to 88.9% in the three emergences of *Cotesia* we sampled (Table 3). In 2003 and 2004, 315 (83.8%) of the 376 individual cocoons present in the collected masses produced secondary (or tertiary) parasitoids, while the remaining 61

Table 3. Incidence of pseudohyperparasitism in 62 masses of cocoons of *Cotesia* sp. D found in the wild in 2002–2004.

| | No. cocoon masses | % masses parasitized | No. cocoons in masses | % cocoons parasitized |
|--------------------|-------------------|----------------------|-----------------------|-----------------------|
| 2002, early spring | 8 | 37.5 | – | – |
| 2002, late spring | 9 | 66.7 | – | – |
| 2003, early spring | 14 | 42.9 | 9 | 88.9 |
| 2003, late spring | 22 | 77.3 | 343 | 83.4 |
| 2004, late spring | 9 | 33.3 | 24 | 87.5 |
| Total | 62 | 56.5 | 376 | 83.8 |

The number of *Cotesia* sp. D adults that emerged from cocoon masses was not recorded in 2002.

cocoons (16.2%) produced *Cotesia* sp. D (this count included adults, dead pupae and dead pharate adults).

The 13 species of secondary (or tertiary) parasitoids identified from *Cotesia* sp. D cocoons are summarized in Table 4. With the probable exception of cases involving *Neochrysocharis albiscapus*, all the species recorded can act as tertiary parasitoids; that is, as parasitoids of pseudohyperparasitoids. This means that numbers of each species might not merely reflect a situation of interspecific competition for the same resource but a much more complex relationship of parasitism between them. Discovery of cocoon masses by multiple species (presumably correlated with their exposure time) was high: only 20 of the 35 parasitized cocoon masses (57.1%) were parasitized by a single species of pseudohyperparasitoid, while eight masses yielded two species, five masses yielded three, and two separate masses yielded four and five species each. There was also a positive correlation between the number of species of pseudohyperparasitoids and the number of cocoons in a mass ($r=0.69$, $p<0.0001$, $n=23$ cocoon masses).

The gregarious eulophid *Neochrysocharis albiscapus* was the commonest pseudohyperparasitoid, being recorded from 29.0% of the collected cocoon masses and 51.4% of the individual cocoons that suffered parasitism. Cocoons of *Cotesia* sp. D that were parasitized by this species produced a mean of 3.9 individuals, with a slightly female orientated sex ratio of 37%:63% (male:female). No incidences of tertiary parasitism involving *N. albiscapus* were found.

Although *Gelis carbonarius* was the second most frequently recorded species, its highly male orientated sex ratio of 87%:13% suggests that it is better adapted to larger cocoons. Moreover, with the exceptions of *Eupelmus atropurpureus* and *Catolaccus ater*, the other species listed in Table 4 were recorded in low numbers and at low frequency, and their degree of association with the *Euphydryas* spp. – *Cotesia* sp. D system is probably similarly rather weak.

Pupal parasitoids

Nine different species of parasitoids were recorded from a total of 66 parasitized pupae of *E. aurinia* and *E. desfontainii* (Table 5). Of these, five species were true and obligatory pupal parasitoids, while the tachinid *Erycia furibunda* was a larva–pupal parasitoid (see above), and the two *Eupelmus* species were facultative generalist primary or secondary parasitoids (both were also reared from *Cotesia* cocoons) that might have been acting as pseudohyperparasitoids. The single specimen of *Agrothereutes parvulus* was reared from a pupa which was also parasitized by *Pteromalus puparum*, but there is no way of telling which species was there first. Except for *Ichneumon stenocerus* (for which the present rearing from *E. aurinia* is the only host record known to us), all the other pupal parasitoids are generalists using hosts other than Melitaeini (Shaw et al. in press).

Levels of pupal parasitism were highly variable between years, ranging from 0–10% to 37–50% of the monitored pupae (Table 5). Of the parasitized pupae (excluding those that produced *Erycia furibunda*), 60.3% were killed by *Pteromalus puparum*, a generalist species known to attack the pupae of a wide range of butterflies (Shaw et al. in press). *Pteromalus puparum* appeared in 4 of the 5 years (although in the single year when it was not recorded the sample consisted of only two pupae), and represented, by far, the species of parasitoid causing the greatest

Table 4. Species of secondary parasitoids recorded from 424 cocoons of *Cotesia* sp. D found in association with host remains (*Euphydryas aurinia* and *Euphydryas desfontainii*) in three consecutive years (2002–2004).

| Species | Family | Indiv./cocoons parasitized | sex ratio (m/f) | No. of cocoon masses | No. of other species in cocoon masses | | | | |
|-----------------------------------|---------------|----------------------------|-----------------|----------------------|---------------------------------------|---|---|---|---|
| | | | | | 0 | 1 | 2 | 3 | 4 |
| <i>Acrolyta?</i> sp. | Ichneumonidae | 1/1 | 1/0 | 1 | | | | | 1 |
| <i>Gelis carbonarius</i> | Ichneumonidae | 59/59 | 48/7 | 15 | 7 | 2 | 4 | 1 | 1 |
| <i>Gelis liparae</i> | Ichneumonidae | 2/2 | 0/1 | 1 | 1 | | | | |
| <i>Gelis proximus</i> | Ichneumonidae | 7/7 | 6/1 | 3 | 1 | 1 | | | 1 |
| <i>Lysibia tenax</i> | Ichneumonidae | 5/5 | 4/0 | 1 | | 1 | | | |
| <i>Brachymeria secundaria</i> | Chalcididae | 4/4 | 4/0 | 1 | | | | | 1 |
| <i>Hockeria unicolor</i> | Chalcididae | 1/1 | 1/0 | 1 | 1 | | | | |
| <i>Neochrysocharis albiscapus</i> | Eulophidae | 831/211 | 279/470 | 18 | 3 | 8 | 5 | 1 | 1 |
| <i>Eupelmus atropurpureus</i> | Eupelmidae | 26/26 | 14/10 | 5 | 1 | 1 | 2 | 1 | |
| <i>Eupelmus vesicularis</i> | Eupelmidae | 6/6 | 1/3 | 2 | 1 | | 1 | | |
| <i>Catolaccus ater</i> | Pteromalidae | 21/21 | 11/7 | 7 | | 3 | 3 | 1 | |
| <i>Pteromalus chrysos</i> | Pteromalidae | 11/11 | 3/7 | 4 | 4 | | | | |
| <i>Pteromalus semotus</i> | Pteromalidae | 1/1 | 0/1 | 1 | 1 | | | | |

Note that some of the parasitoids that were counted could not be sexed.

Table 5. Parasitoids recorded from pupae of *Euphydryas aurinia* (*aur*) and *Euphydryas desfontainii* (*des*) placed experimentally in the field, and from wild pupae (in bold type).

| Species | Family | 2002 | | | 2003 | | | 2004 | | | 2005 indet. | 2006 <i>aur</i> | No. pupae parasitized |
|---|---------------|------------|------------|----------|------------|------------|----------|------------|------------|----------|----------------|--------------------|--------------------------|
| | | <i>aur</i> | <i>des</i> | indet. | <i>aur</i> | <i>des</i> | indet. | <i>aur</i> | <i>des</i> | indet. | | | |
| <i>Apechthys punctator</i> | Ichneumonidae | | | | 3 | 2 | 3 | 1 | | 1 | | 4 | 14 |
| <i>Pimpla rufipes</i> | Ichneumonidae | | | | 1 | | | | | | | | 1 |
| <i>Ichneumon stenocerus</i> | Ichneumonidae | | | | | | | | | 1 | | | 1 |
| <i>Agrothereutes parvulus</i> * | Ichneumonidae | | | | (1) | | | | | | | | (1) |
| <i>Brachymeria tibialis</i> | Chalcididae | | | | | | 1 | | | 1 | 2 | 1 | 5 |
| <i>Eupelmus atropurpureus</i> | Eupelmidae | | | | | | 1 | | | | | | 1 |
| <i>Eupelmus vesicularius</i> | Eupelmidae | | | | | | | | | | | 1 | 1 |
| <i>Pteromalus puparum</i> | Pteromalidae | | 2 | 1 | 21 | 2 | 5 | | | | | | 35 |
| <i>Erycia furibunda</i> † | Tachinidae | 3 | | | 3 | | | | | | | | 8 |
| Pupae parasitized/ pupae not disappearing | | 0/27 | 2/20 | – | 25/67 | 4/8 | – | 1/42 | 0/27 | – | – | – | 66 |

**Agrothereutes parvulus* was reared from a pupa that was also parasitized by *Pteromalus puparum*.

†*Erycia furibunda* is not a true pupal parasitoid but a larva–pupal parasitoid.

impact on pupal mortality (Table 5). Second in importance was *Apechthis compunctor*, another generalist parasitoid known to attack a broad range of especially butterfly pupae (Shaw et al. in press). It appeared in 3 of the 5 years, causing 24.1% of the recorded losses attributed to pupal parasitism. *Brachymeria tibialis* did not appear in the main pupal experiments, but it was recorded regularly from wild pupae and also from one pupa placed in the field in 2006. The other species of parasitoids can probably be considered peripheral because they appeared only on single occasions in the host populations.

Discussion

The 5-year investigation reported here has shown the extent of the parasitoid complexes attacking coexisting populations of *E. aurinia* and *E. desfontainii* in a Spanish site. These complexes consist of at least three primary larval parasitoids and five primary pupal parasitoids, plus a diverse array of secondary and facultatively tertiary parasitoids associated with the cocoons of *Cotesia* sp. D. Notably, not a single case of egg parasitism was recorded. Although egg parasitoids might have been overlooked owing to the relatively small sample size, lack of egg parasitism seems to be common in populations of species of Melitaeini that have been studied in detail (van Nouhuys and Hanski 2004; but see Drummond et al. 1970; Stamp 1981).

Other studies carried out in northern Europe and central Asia have provided similar, albeit more simplified, pictures of the parasitoid community associated with *E. aurinia* (England: Porter 1981; Finland: Komonen 1997; Buryatia, in Russia: Wahlberg et al. 2001; Sweden: Eliasson and Shaw, 2003). An outstanding feature in all the *E. aurinia* systems investigated is the presence of a specialist larval parasitoid belonging to the genus *Cotesia* (either a species from the *C. melitaeorum* agg., or *C. bignellii*). A second larval specialist belonging to *Erycia* (either *Erycia fatua* or, more usually, *Erycia furibunda*) may be present in some host populations, but missing in others (e.g. in some intensively studied British populations: O.T. Lewis, personal communication). Co-occurrence of these two larval parasitoids may lead to interspecific competition between them, in a similar manner as has been described for *C. melitaeorum* agg. and *Hyposoter horticola* parasitizing Finnish populations of *Melitaea cinxia* (van Nouhuys and Hanski 2004), but we are unable to offer data to support this.

The studies so far available provide very few data on pupal parasitoids of *E. aurinia*. Komonen (1997) only mentions *Ichneumon gracilicornis*, a species known to attack pupae of a wide range of Nymphalidae (Heliconiinae, Nymphalinae and Satyrinae) (Hinz and Horstmann 2007), while several records from various parts of Europe indicate that *Pteromalus apum* frequently uses *E. aurinia* as a host (Askew and Shaw 1997; Shaw 2002) rather than the more common *Pteromalus puparum* (this study). The higher diversity of pupal parasitoids found in our Spanish population probably merely reflects more thorough sampling. The difficulty of locating pupae in the field means that an experimental approach, as followed here, is necessary to obtain a more comprehensive understanding of *Euphydryas* pupal parasitoids.

From the whole set of primary parasitoids recorded in our study, it seems that only *Cotesia* sp. D, *Erycia furibunda* and, possibly, *Ichneumon stenoceros* are true specialists with very narrow host ranges, and even the poorly known *Ichneumon stenoceros* was recorded only from a single pupa and conceivably may more often

use other hosts in the area. The remaining species, which attack mainly the host pupal stage, are known to be broad generalist parasitoids (e.g. Shaw et al. in press). This finding, which contrasts with the high specialization seen in the most important of the larval parasitoids, would seem to contradict the hypothesis that generalist predators and parasitoids should be deterred from attacking Melitaeini butterflies by the toxic secondary compounds (iridoids) sequestered by the larvae (Bowers 1980, 1981).

As was to be expected from the known representation of parasitoids in the natural enemy complexes of Melitaeini butterflies (van Nouhuys and Hanski 2004), we found heavy mortality induced by parasitoids in our study (up to about 15% due to larval parasitism and 50% due to pupal parasitoids), though not as high a rate of parasitism by *Cotesia* as that recorded for *E. aurinia* in England (Porter 1983) or for *M. cinxia* in Finland (Lei et al. 1997). However, our figures for both larval and pupal parasitism are likely to be substantial underestimates. For larval parasitism our strenuous avoidance of solitary mature larvae (aimed at ensuring that we did not overestimate parasitism in the second postdiapause cohort) undoubtedly resulted in underestimation, although by a factor that is impossible to estimate. Also, the number of larval groups parasitized (Table 1) might have been underestimated through sampling only a small fraction of larvae from each group. Pupal parasitism might have been underestimated by our technique of placing (in part) 1-day-old pupae in the field because some parasitoids (e.g. *Pteromalus* and *Ichneumon* species) seek the host as it goes to pupate, and probably find it best before it is actually a pupa [and, because they are believed to detect the host via chemoreception of moulting fluids, certainly by 1 day later the hosts will have lost some of their attraction; see Hinz (1983)]. The possibility that our sampling might have taken place at an atypical point during some kind of fluctuation that remains uninvestigated should also be recognized.

However, our study also showed that *Cotesia* sp. D was subjected to very heavy secondary parasitism during three consecutive seasons (Table 3), a factor that may keep this parasitoid's population small most of the time, thereby reducing its impact on the butterfly hosts. Van Nouhuys and Tay (2001) found that population sizes of *C. melitaeorum* agg. depending on Finnish *M. cinxia* always remain small owing to the severe impact of generalist predators and secondary parasitoids such as *Gelis* spp. It was shown that generalist enemies behaved in a density-dependent manner, thereby playing a direct role in regulating parasitoid population size. A similar regulatory effect may occur in our system as indicated by the positive correlation that exists between the number of recorded pseudohyperparasitoid species and the number of *Cotesia* sp. D cocoons in a mass. In any case, the impact of pseudohyperparasitism on the *Cotesia* sp. D population is substantial and this should be taken into account if any population modelling involving *Euphydryas*–*Cotesia* is to be meaningful. There are no reasons to believe that our study system is exceptional in this respect, and this conclusion should extend to other Melitaeini and their *Cotesia* parasitoids.

Most of the parasitoids were recorded from both of the two *Euphydryas* species. This was true not only for the generalist pupal parasitoids that were reared in numbers but also for the *Cotesia* specialist, a genus which, when attacking Melitaeini butterflies, is known to consist of an aggregate of cryptic species showing extreme host specialization (Kankare and Shaw 2004; Kankare et al. 2005a,b). Moreover,

Erycia furibunda, the other specialist larval (strictly, larva–pupal in this case) parasitoid which in our study was confirmed only from *E. aurinia*, can also use *E. desfontainii* as a host (Ford et al. 2000).

Indiscriminate use of *E. aurinia* or *E. desfontainii* by *Cotesia* sp. D is suggested by the similar rate of parasitism of the two host species in a given season, both between and within larval groups (Table 1). Although the use of different host plants by the two *Euphydryas* species rules out the possibility of direct competitive interactions among them, the striking coincidence in their parasitoid complexes makes highly likely a phenomenon of apparent competition between hosts sharing common natural enemies (Holt and Lawton 1993; Lewis et al. 2002). The existence of several co-occurring populations of both butterfly species, in highly asymmetrical abundance combinations around the studied area (J. Planas, personal observation), would provide a good opportunity for experimental manipulation and field testing of this hypothesis (cf. Chaneton and Bonsall 2000).

Another area for further investigation is the pattern of interactions among the guild of secondary parasitoids exploiting the resource provided by *Cotesia* cocoons. Knowledge of the natural history of the species identified suggests that these interactions may include not only interspecific competition for a common resource, but also more complex interactions among species acting potentially as tertiary parasitoids with different degrees of preference. One very striking finding was that the gregarious species *Neochrysocharis albiscapus* appears to escape from being parasitized by the other pseudohyperparasitoids. This suggests a strong selective advantage of gregariousness for *Neochrysocharis albiscapus*, which appears to have a strong or even exclusive association with cocoons of microgastrine Braconidae such as *Cotesia* (cf. Universal Chalcidoidea Database 2007), in an evolutionary context in which most other pseudohyperparasitoids are adapted as solitary parasitoids in *Cotesia*-sized (or larger) cocoons. That the small size of *Neochrysocharis albiscapus* individuals is below the resource viability threshold for other pseudohyperparasitoids can be deduced from the fact that all of the other pseudohyperparasitoids reared were substantially larger.

Acknowledgements

We thank Saskya van Nouhuys for comments on earlier versions of the manuscript, and Dick Askew, Klaus Horstmann, Heinz Schnee, Martin Schwarz and Hans-Peter Tschorsnig for their help in the identification of some of the material collected in this study.

References

- Askew RR, Shaw MR. 1997. *Pteromalus apum* (Retzius) and other pteromalid (Hym.) primary parasitoids of butterfly pupae in western Europe, with a key. *Entomol Mthly Mag.* 133:67–72.
- Bowers MD. 1980. Unpalatability as a defense strategy of *Euphydryas phaeton* (Lepidoptera: Nymphalidae). *Evolution.* 34:586–600.
- Bowers MD. 1981. Unpalatability as a defense strategy of western checkerspot butterflies (*Euphydryas* Scudder, Nymphalidae). *Evolution.* 35:367–375.
- Chaneton EJ, Bonsall MB. 2000. Enemy-mediated apparent competition: empirical patterns and the evidence. *Oikos.* 88:380–394.

- Drummond BAI, Bush GL, Emmel TC. 1970. The biology and laboratory culture of *Chlosyne lacinia* Geyer (Nymphalidae). *J Lepidopt Soc.* 24:135–142.
- Ehrlich PR, Hanski I. 2004. On the wings of checkerspots. A model system for population biology. Oxford (UK): Oxford University Press.
- Eliasson CU, Shaw MR. 2003. Prolonged life cycles, oviposition sites, foodplants and *Cotesia* parasitoids of Melitaeini butterflies in Sweden. *Oedippus.* 21:1–52.
- Ford HD, Ford EB. 1930. Fluctuation in numbers and its influence on variation in *Melitaea aurinia*, Rott. (Lepidoptera). *Trans Entomol Soc Lond.* 78:345–351.
- Ford TH, Shaw MR, Robertson DM. 2000. Further host records of some west Palearctic tachinidae (Diptera). *Entomol Rec J Variat.* 112:25–36.
- Hawkins BA, Sheehan W (eds). 1994. Parasitoid community ecology. Oxford (UK): Oxford University Press.
- Hinz R. 1983. The biology of the European species of the genus *Ichneumon* and related species (Hymenoptera: Ichneumonidae). *Contrib Am Entomol Inst.* 20:151–152.
- Hinz R, Horstmann K. 2007. Über Wirtsbeziehungen europäischer *Ichneumon*-Arten (Insecta, Hymenoptera, Ichneumonidae, Ichneumoninae). *Spixiana.* 30:39–63.
- Holt RD, Lawton JH. 1993. Apparent competition and enemy-free space in insect host-parasitoid communities. *Am Nat.* 142:623–645.
- Kankare M, Shaw MR. 2004. Molecular phylogeny of *Cotesia* Cameron, 1891 (Insecta: Hymenoptera: Braconidae: Microgastriinae) parasitoids associated with Melitaeini butterflies (Insecta: Lepidoptera: Nymphalidae: Melitaeini). *Mol Phylogen Evol.* 32:207–220.
- Kankare M, Stefanescu C, van Nouhuys S, Shaw MR. 2005a. Host specialization by *Cotesia* wasps (Hymenoptera: Braconidae) parasitizing species-rich Melitaeini (Lepidoptera: Nymphalidae) communities in north-eastern Spain. *Biol J Linn Soc.* 86:45–65.
- Kankare M, van Nouhuys S, Hanski I. 2005b. Genetic divergence among host-specific cryptic species in *Cotesia melitaeorum* aggregate (Hymenoptera: Braconidae), parasitoids of checkerspot butterflies. *Ann Entomol Soc Am.* 98:382–394.
- Komonen A. 1997. The parasitoid complexes attacking Finnish populations of two threatened butterflies, *Euphydryas maturna* and *E. aurinia*. *Baptria.* 22:105–109.
- Komonen A. 1998. Host species used in parasitoids of Melitaeini butterflies in southern France. *Baptria.* 23:194–200.
- Lei GC, 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.
- Lewis OT, Memmott J, Lasalle J, Lyal CHC, Whiteford C, Godfray HCJ. 2002. Structure of a diverse tropical forest insect–parasitoid community. *J Anim Ecol.* 71:855–873.
- Moore SD. 1989. Patterns of juvenile mortality within an oligophagous insect population. *Ecology.* 70:1726–1737.
- Morris RJ, Lewis OT, Godfray HCJ. 2004. Experimental evidence for apparent competition in a tropical forest food web. *Nature.* 393:310–313.
- Morris RJ, Müller CB, Godfray HCJ. 2001. Field experiments testing for apparent competition between primary parasitoids mediated by secondary parasitoids. *J Anim Ecol.* 70:301–309.
- Müller CB, Adriaanse ICT, Belshaw R, Godfray HCJ. 1999. The structure of an aphid–parasitoid community. *J Anim Ecol.* 68:346–370.
- Porter K. 1981. The population dynamics of small colonies of the butterfly *Euphydryas aurinia* [D. Phil. Thesis]. Oxford (UK): University of Oxford.
- Porter K. 1983. Multivoltinism in *Apanteles bignellii* and the influence of weather on synchronisation with its host *Euphydryas aurinia*. *Entomol exp appl.* 34:155–162.
- Shapiro AM. 1976. Beau Geste? *Am Nat.* 110:900–902.
- Shaw MR. 2002. Experimental confirmation that *Pteromalus apum* (Retzius) (Hym., Pteromalidae) parasitizes both leaf-cutter bees (Hym., Megachilidae) and fritillary butterflies (Lep., Nymphalidae). *Entomol Mthly Mag.* 138:37–41.

- Shaw MR, Stefanescu C, van Nouhuys S. in press. Parasitoids of European butterflies (Heperioidea and Papilionoidea). In: Settele J, Shreeve T, Konvicka M, van Dyck H, editors. Ecology of butterflies in Europe. Cambridge (UK): Cambridge University Press.
- Singer MC, Stefanescu C, Pen I. 2002. When random sampling does not work: standard design falsely indicates maladaptive host preferences in a butterfly. *Ecol Lett.* 5:1–6.
- Stamp NE. 1981. Parasitism of single and multiple egg clusters of *Euphydryas phaeton* (Nymphalidae). *N Y Entomol Soc.* 89:89–97.
- Stamp NE. 1984. Interactions of parasitoids and checkerspot caterpillars *Euphydryas* spp. (Nymphalidae). *J Res Lepidopt.* 23:2–18.
- Stefanescu C, Peñuelas J, Sardans J, Filella I. 2006. Females of the specialist butterfly *Euphydryas aurinia* (Nymphalinae: Melitaeini) select the greenest leaves of *Lonicera implexa* (Caprifoliaceae) for oviposition. *Eur J Entomol.* 103:569–574.
- Universal Chalcidoidea Database [Internet]. The Natural History Museum [cited 2007 November 20]; Available from: <http://www.nhm.ac.uk/research-curation/projects/chalcidoids>.
- van Nouhuys S, Hanski I. 2000. Apparent competition between parasitoids mediated by a shared hyperparasitoid. *Ecol Lett.* 3:82–84.
- van Nouhuys S, Hanski I. 2004. Natural enemies of checkerspot butterflies. In: Ehrlich PR, Hanski I, editors. On the wings of checkerspots: a model system for population biology. Oxford (UK): Oxford University Press. p. 161–180.
- van Nouhuys S, Hanski I. 2005. Metacommunities of butterflies, their host plants and their parasitoids. In: Holyoak M, Leibold MA, Holt RD, editors. Metacommunities: spatial dynamics and ecological communities. Chicago (IL): University of Chicago Press. p. 99–121.
- van Nouhuys S, Tay WT. 2001. Causes and consequences of mortality in small populations of a parasitoid wasp in a fragmented landscape. *Oecologia.* 128:126–133.
- Waage JK, Greathead D, editors, 1986. Insect parasitoids. 13th Symposium of the Royal Entomological Society. London (UK): Academic Press.
- Wahlberg N, Kullberg J, Hanski I. 2001. Natural history of some Siberian melitaeine butterfly species (Melitaeini: Nymphalidae) and their parasitoids. *Entomol Fenn.* 12:72–77.
- White RR. 1986. Pupal mortality in the Bay checkerspot butterfly (Lepidoptera: Nymphalidae). *J Res Lepidopt.* 25:52–62.